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JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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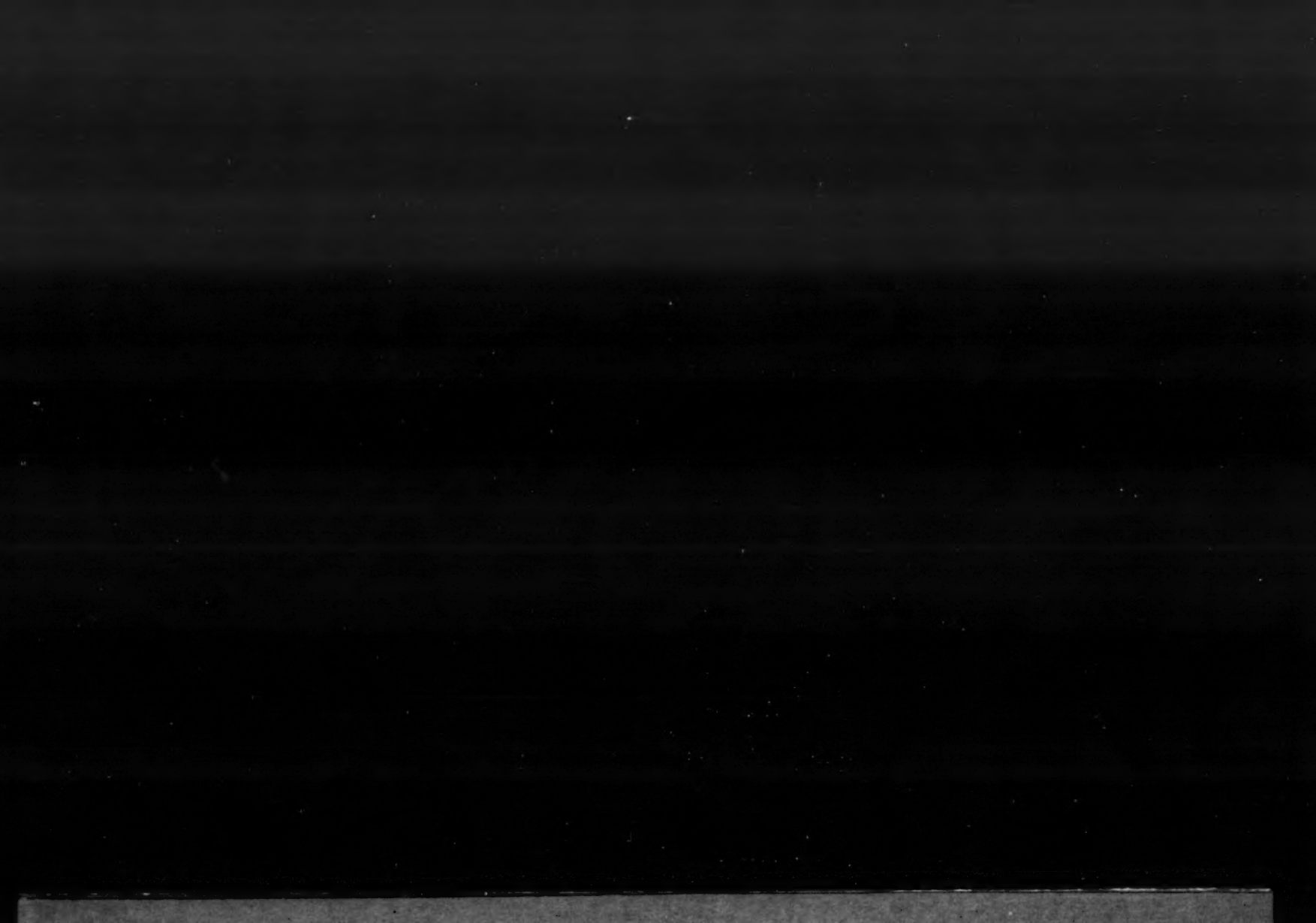

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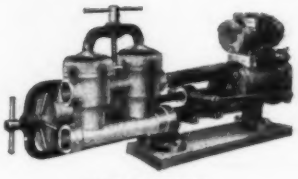

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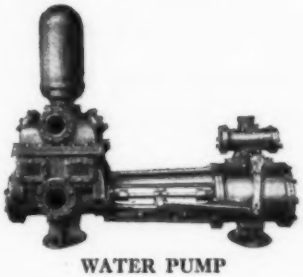

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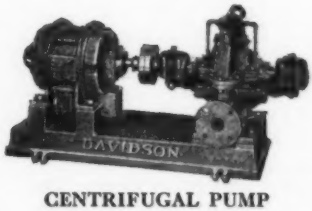

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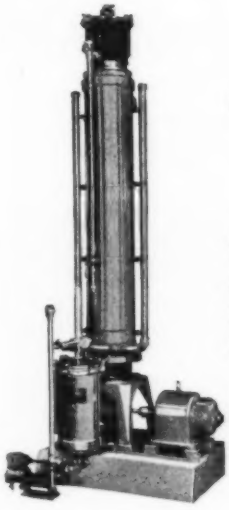

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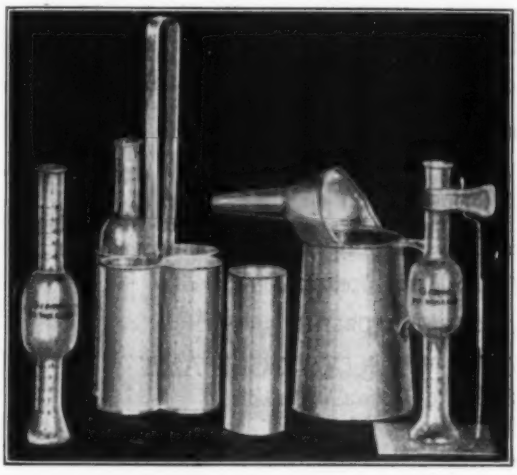

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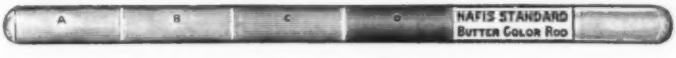

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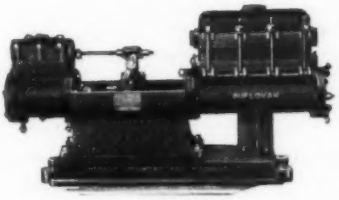

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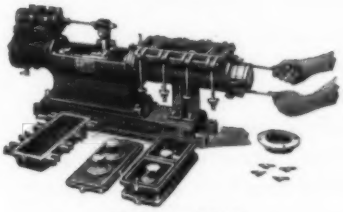

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ENERGY REQUIREMENTS OF DAIRY COWS

I. THE QUANTITIES OF NUTRITIVE ENERGY NECESSARY TO MAINTAIN DAIRY COWS IN NUTRITIVE EQUILIBRIUM*

EDWARD B. MEIGS AND H. T. CONVERSE

Research Laboratories, Bureau of Dairying, United States Department of Agriculture

INTRODUCTION

For the past eight years, work on the nutriton of dairy cows has been in progress at the Beltsville (Md.) experiment farm of the United States Department of Agriculture. In the carrying out of this work it has been necessary, as it always is in scientific research, to center on certain aspects of the subject. But the effort has been made to keep, at the same time, more or less in touch with the whole field, in the hope that it would be possible to make some progress toward working out rations which would be permanently satisfactory for dairy cows—rations, that is, on which a liberal milk yield with satisfactory general health and reproductive capacity could be maintained in a herd of dairy cows through an unlimited number of generations.

The working out of such rations brings up quantitative as well as qualitative questions; and, if one surveys this field of nutrition as it stands at the present time, one finds that only two of the numerous dietary factors have so far been investigated from the quantitative standpoint, namely, protein and nutritive energy.[1]

Recent developments in physiological chemistry have shown that there is, strictly speaking, no such thing as a quantitative requirement of protein for dairy cows. Different proteins differ widely in their chemical constitution, and the dairy cow requires much larger quantities of some of them for the manufacture of a given quantity of milk protein than of others.

* Received for publication January 15, 1925.

[1] There is a tacit disagreement in the literature as to how the energy content of foods should be measured; some investigators give their feeding standards in terms of "total digestible nutrients"; others, in terms of "net energy." Throughout this article the phrase "nutritive energy" will be used to cover either of these conceptions.

With regard to nutritive energy, on the other hand, there is reason to believe that the quantity required by dairy cows under different conditions of milk and fat yield and of body weight can be at least approximately determined; and, as this question stands at the very center of any general plan for rationing these animals, records have been kept at Beltsville of the food eaten, of the milk and fat yield, and of the changes in body weight of the members of the herd, which, it is believed, will throw light on it. The results are to be regarded as supplementing those which have already been obtained by other investigators in the same field, and must be considered and discussed in close connection with them.

EXISTING FEEDING STANDARDS

The experimental work on which the existing feeding standards are based is of three types. Haecker (3, 4, 5) and Eckles (2) determined the quantities of food necessary to keep milking cows at uniform body weight. Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a milking cow (1, p. 493–500, 511–516).[2] Savage (7) raises the question: What will happen if cows are fed more than is necessary, according to Haecker's results, to keep them at uniform body weight. For the present, the question raised by Savage will be left aside, and the discussion will be confined to the question how much food is necessary to keep milking cows in nutritive equilibrium or at uniform body weight.

Even at this early stage of the subject one encounters more than one tacit difference of opinion among the different investigators. Eckles used Armsby's tables to obtain the net energy content of the feeds required by his cows for the maintenance of uniform body weight and gave his standard for the energy requirements of dairy cows in terms of net energy. Some years later, Armsby published a feeding standard for dairy cows. The energy requirements in Armsby's standard are given in terms of

[2] Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average.

net energy, and are based, as was stated above, on one of Kellner's experiments. If one compares Armsby's standard with Eckles's, one finds that the former requires from 11 to 23 per cent less net energy than the latter for a given amount of milk.

In Eckles's experiments the food intake, milk and fat yield, and body weight were followed in eight milking cows for a year each, and in two others for 110 and 120 days respectively; in the experiment of Kellner, on which Armsby's standard is based, the complete energy balance was determined in the case of a single milking cow for a period of two weeks. It may be said, on the one hand, in explanation of the disagreement between the two standards, that the maintenance of uniform body weight is not always a very sure indication that an animal is in nutritive equilibrium; on the other hand, it may be said that it is a little unsafe to base a feeding standard which is to be used in practice on the results of a single physiological experiment.

Changes in body weight in mammals are caused chiefly by changes in the quantity of muscle and fat contained in their bodies. Changes in other tissues are likely to be so small as to be masked by changes occurring in the two above mentioned. Muscle is a tissue which contains from seventy to eighty per cent of water; its dry matter is chiefly protein. Fat, on the other hand, contains very little water, and its dry matter has about $2\frac{1}{4}$ times as much nutritive energy per gram as protein. It may be considered, therefore, that a gram of fat tissue has from eight to ten times as much nutritive energy as a gram of muscle tissue. If an animal is fed a ration which contains a surplus of protein (as in Eckles's experiments), though a slight deficit in nutritive energy, it is quite conceivable that it might build muscle tissue from the protein surplus and supply the deficit in nutritive energy by using a small quantity of its own body fat. Under these circumstances, the animal might gain weight though being in negative energy balance. It has been shown experimentally that this process actually does occur in young animals when they are fed rations deficient in nutritive energy (1, p. 280).

The fact that an animal is gaining weight does not, therefore, necessarily show that it is receiving sufficient nutritive energy

in its rations. But if a healthy animal, which is getting a surplus of protein and of the other chemical compounds necessary for complete nutrition, is losing weight, this does show that it is not receiving sufficient nutritive energy in its rations. For the surplus of protein and other nutritive compounds will prevent it from losing muscle tissue, except so far as such loss may be necessary to supply the energy deficiency; and, if this deficiency does not exist, neither muscle nor fat tissue will be lost.

The case may be summed up by saying that if a healthy animal which is receiving a well-balanced ration with a liberal protein content loses weight, the fact constitutes strong evidence for believing that the ration used is deficient in nutritive energy. Conversely, a well-balanced ration high in protein which is just sufficient to maintain a healthy animal at uniform body weight certainly does not contain a surplus of nutritive energy. In the case of a short-time experiment with a young animal, such a ration might be somewhat deficient in nutritive energy for the reasons above mentioned, but the process of building muscle tissue at the expense of body fat could not go on very long. There is every reason to believe, therefore, that uniform body weight maintained for a year or more is a very sure indication that the animal under consideration remained nearly in nutritive equilibrium throughout the period of the experiment. If the animal under consideration was mature and farrow, and had, therefore, no tendency to build watery tissues at the expense of body fat, the reasons for thinking that it was in nutritive equilibrium are still stronger. It seems to us, therefore, that such experiments as those of Eckles[3] furnish a very good basis for judging the average quantities of food necessary to keep milking cows in nutritive equilibrium under practical conditions.

RESULTS OBTAINED AT THE BELTSVILLE FARM ON THE QUANTITY OF NUTRITIVE ENERGY NECESSARY TO MAINTAIN UNIFORM BODY WEIGHT

The records which have been kept at the Beltsville farm for the last four years make it possible to throw still more light on

[3] The cows used by Eckles were mature and farrow.

the relation between food intake and change in body weight in milking cows. These records consist of figures for the monthly milk and fat yield, food consumption, and change in body weight for every member of the herd. It is possible from these records to calculate the monthly intake of either net energy or total nutrients for any cow and to compare it with her requirements according to any of the feeding standards.[4] It is easy then to compare the surplus or deficit in nutritive energy consumed with the monthly change in body weight, and, in this way, to determine which of the standards comes nearest to indicating the quantity of nutritive energy required to maintain uniform body weight.

The body weight of the cows at Beltsville has been determined by weighing them on three successive days at the same time each day. The average of these three weights is taken as the body weight of the cow at the period of the weighings. Body weights were usually taken at the beginning of each calendar month; in calculating the maintenance requirements for any given month, the average between the figure obtained at the beginning of the month and that obtained at the beginning of the next succeeding month was taken.

In the Armsby, Eckles, and Savage feeding standards, the maintenance requirements are given for 1000 pound cows. In calculating the requirements for cows that weighed more or less than a thousand pounds we used corrections based on the assumption that maintenance requirements are proportional to body surface, our requirements being calculated from the formula

$$X = M\left(\frac{W}{1000}\right)^{\frac{2}{3}}$$

where X is the maintenance requirement to be determined; M, the maintenance requirement of a 1000-pound cow; and W, the body weight of the cow whose requirement is to be determined.

For the purposes of this study the cows at the Station may be divided into two or three different classes. Each year a number

[4] Of course none of the cows used in these studies had any pasture.

of them are run on yearly official test. They are kept in box stalls, milked three times a day, and fed very liberally on a ration with a rather narrow nutritive ratio, not only while they are milking, but also before they calve. Another set of cows has been used in experiments which required that they should be fed as much as they would eat while milking. They also received a ration with a rather narrow nutritive ratio. They were fed liberally before they calved, though not so high as the test cows; and they were kept in stanchions and milked only twice a day. Still a third set of cows has been kept under the ordinary herd routine. They have been kept in stanchions and milked only twice a day; and, while milking, were fed decidedly less liberally than either of the other sets. Before calving, they were fed about as liberally as the second-mentioned group. Their ration had a somewhat wider nutritive ratio than that of the other two sets. In making the study at present under discussion, the herd cows were put in one group, and all the others in a second group. As young cows and pregnant cows are likely to be building watery tissues at the expense of body fat, only those animals have been used which were over five years old and farrow.

It has been thought worth while to compare consumption with requirement and change in body weight according to the Armsby, Eckles, and Savage feeding standards;[5] and the data contained in the records have been handled in the following manner. The quantity of net energy or of total digestible nutrients required by each cow in each calendar month for her maintenance and milk and fat yield, according to each of the above-mentioned standards, has been calculated and subtracted from her consumption of either net energy or total digestible nutrients. The percentage consumed above or below the requirement was then calculated. For each of the standards, then, each group of cows, as above

[5] In calculating the net energy content of dairy feeds, we have throughout used the figures given in Table VII in Armsby's "Nutrition of Farm Animals" (1, p. 715–721). In calculating the total digestible nutrients, we have used the figures from Table III of Henry and Morrison's "Feeds and Feeding" (6, p. 653–666). In calculating requirements for maintenance and milk yield we have used Table I, p. 711, and Table V, p. 714, of Armsby's book (1), and the tables given on p. 133 of "Feeds and Feeding" (6).

described, was divided into two subgroups, namely, those that ate less than they required, and those that ate more. The average percentage consumed above or below requirement, for each of these groups, was then calculated and compared with the average corresponding monthly change in body weight.

The results of these calculations are given in tables 1 and 2. It will be seen from the figures given in the tables that there was a marked general tendency for the cows to lose weight when they were eating less than the requirements of either the Eckles or the Savage standard, and a very marked tendency for them to lose weight when they were consuming less than the Armsby standard required. But a study of the figures for the individual cow-months shows that these are rather irregular.

It was hoped that our procedure for determining body weight described above would give a fairly good idea of any change that might take place in the weight of the body tissues. The sources of error in this kind of study are changes in the contents of the intestinal tract and the bladder, and changes in the water content of the body tissues. It would seem not unlikely, a priori, that by the procedure above outlined one would get figures which, when corrected by subtracting a certain constant to represent the contents of the intestine and of the bladder, would represent the true weight of the body tissues with their average water content.

A study of the manner in which the body weight of the individual cows varies from month to month shows, however, that this is not always the case. It is not uncommon to find that a cow kept for two or three months on a deficient ration will lose perhaps a hundred pounds in the first part of the period, according to our method of determining the body weight, and then regain half of the previous loss, though still on the same ration. It would seem, therefore, that an abnormally high or low intestinal content or water content of the body tissues may persist for several days, and even our three-day average weights can not be taken as a very sure indication of the weight of the body tissues plus a constant.

These irregularities are readily smoothed out, however, if

TABLE 1

Relation between change in body weight and percentage of net energy or total digestible nutrients consumed above or below the requirements of Armsby, Eckles, and Savage feeding standards

Most of the cows used were under test conditions; all of them were fed very liberally while milking.

ARMSBY STANDARD				ECKLES STANDARD				SAVAGE STANDARD			
Cows eating less than Armsby requirement: individual cases				Cows eating less than Eckles requirement: individual cases				Cows eating less than Savage requirement: individual cases			
Percentage net energy consumed below requirement	Pounds body weight gained or lost in month			Percentage net energy consumed below requirement	Pounds body weight gained or lost in month			Percentage total digestible nutrients consumed below requirement	Pounds body weight gained or lost in month		
15.5	−13	Cows eating more than Armsby requirement: individual cases (continued)		22.1	−13	Cows eating more than Eckles requirement: individual cases (continued)		27.6	−13	Cows eating more than Savage requirement: individual cases	
3.8	−30			11.2	−30			17.9	−30		
				8.7	−118			15.3	−8		
				8.1	−8			13.1	−118		
				5.6	−39			9.5	+35		
				4.8	+23			8.6	+23		
				2.8	0			7.7	−39		
								7.5	−23		
								7.3	0		
Ave. 9.6	−21.5			Ave. 9.0	−26.4			4.3	+51		
								1.3	−32		
								Ave. 8.7	−11.2		
		19.3	+13			9.1	+4			0.8	+11
		19.4	−5			9.5	+2			0.8	+22
		20.8	+27			9.8	+13			0.9	+14
		22.1	−14			9.9	+11			1.2	+75
		22.3	+32			10.3	+22			1.5	+2
		23.0	−1			11.0	+25			1.7	−1
		23.1	+19			11.8	+75			2.6	+1
		23.2	+11			11.9	+1			3.0	+25
		23.2	+22			12.6	+32			3.8	+13
		23.5	+41			13.0	+41			4.3	+27
		23.7	−21			13.6	0			4.5	+32
		24.1	+1			14.5	+19			5.0	−14
		24.8	+4			14.7	+14			5.8	−5
		25.4	−31			15.2	−21			6.0	0
		25.6	0			15.5	−1			6.0	−21
		25.6	+75			16.2	−31			6.2	+14

| Cows eating more than Armsby requirement: individual cases | | |

TABLE 2

Relation between change in body weight and percentage net energy or total digestible nutrients consumed above or below the requirements of the Armsby, Eckles, and Savage feeding standards

The cows used were under herd conditions and not very liberally fed while milking.

Cows eating less than Armsby requirement: individual cases			ARMSBY STANDARD			ECKLES STANDARD			SAVAGE STANDARD		
	Percentage net energy consumed below requirement	Pounds body weight gained or lost in month		Percentage net energy consumed above requirement	Pounds body weight gained or lost in month		Percentage net energy consumed above requirement	Pounds body weight gained or lost in month		Percentage total digestible nutrients consumed below requirements	Pounds body weight gained or lost in month
	29.1	−118	Cows eating more than Armsby requirement: individual cases	0.2	−37	Cows eating less than Eckles requirement: individual cases	35.2	−71	Cows eating more than Eckles requirement: individual cases	3.7	+18	Cows eating less than Savage requirement: individual cases	38.1	−118
	28.3	−71		5.6	−30		33.7	−118		4.2	−29		37.8	−71
	18.2	−56		9.0	+5		25.1	−44		5.7	−17		30.7	−56
	17.5	−44		9.3	−37		24.1	−56		6.0	+15		28.3	−44
	8.8	−74		9.8	+74		23.4	−74		7.2	+32		25.1	−74
	8.1	−52		12.5	+18		19.5	−52		8.4	+5		18.1	−52
	5.3	+19		14.3	+15		15.0	+48		10.7	+29		18.1	+19
	2.0	+48		17.6	−29		12.2	+19		11.0	+11		14.9	+48
				17.8	+32		8.4	−37		19.6	+22		13.5	−37
				19.9	−17		3.3	−30		20.2	+9		12.1	−30
Ave.	14.7	−43.5		25.0	+29		2.9	−37		21.7	+7		7.5	−37
				25.1	+11		1.0	+5					6.0	+5
				26.0	+5		0.7	+74					5.5	+74
				29.2	+22	Ave.	15.8	−28.7		10.8	+9.3		4.4	+18
				32.6	+9								3.0	+15
				39.7	+7								2.0	+32
			Ave.	18.3	+4.8							Ave.	16.6	−19.2

Cows eating more than Savage requirement: individual cases		
	Percentage total digestible nutrients consumed above requirement	Pounds body weight gained or lost in month
	0.6	−29
	1.6	−17
	3.9	+11
	6.2	+29
	7.3	+5
	7.8	+22
	12.1	+7
	12.4	+9
Ave.	6.5	+4.6

enough figures are taken and averaged. For the present, the question of how many figures are necessary to secure a satisfac-

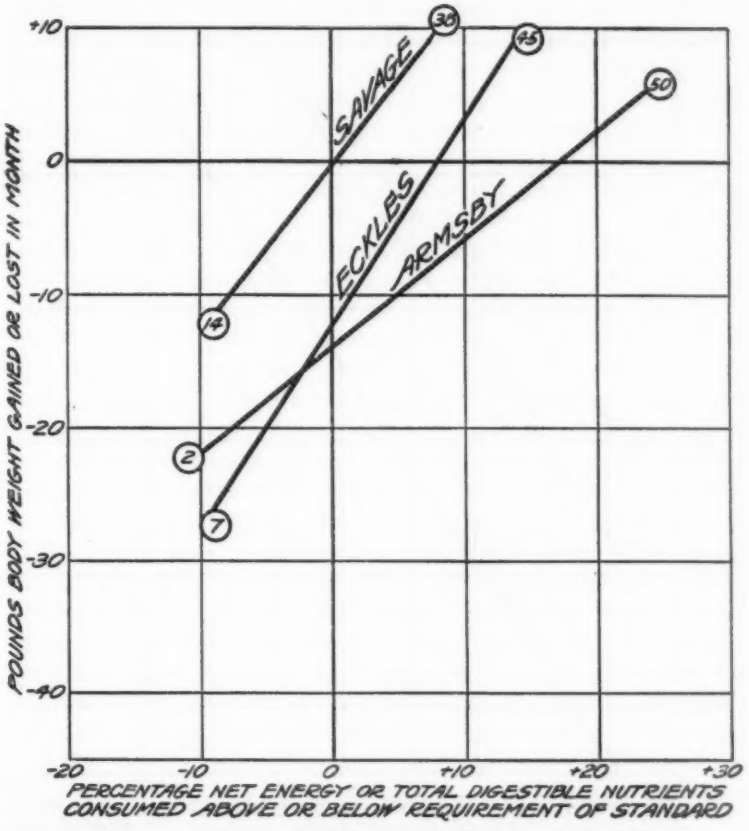

FIG. 1. RELATIONS BETWEEN CHANGE IN BODY WEIGHT AND PERCENTAGE OF NET ENERGY OR TOTAL DIGESTIBLE NUTRIENTS CONSUMED ABOVE OR BELOW REQUIREMENTS OF ARMSBY, ECKLES AND SAVAGE STANDARDS

Graphs plotted from data in table 1

tory average will be left aside, and the results as they have been obtained will be given and discussed.

The results given in tables 1 and 2 may be plotted as in figures

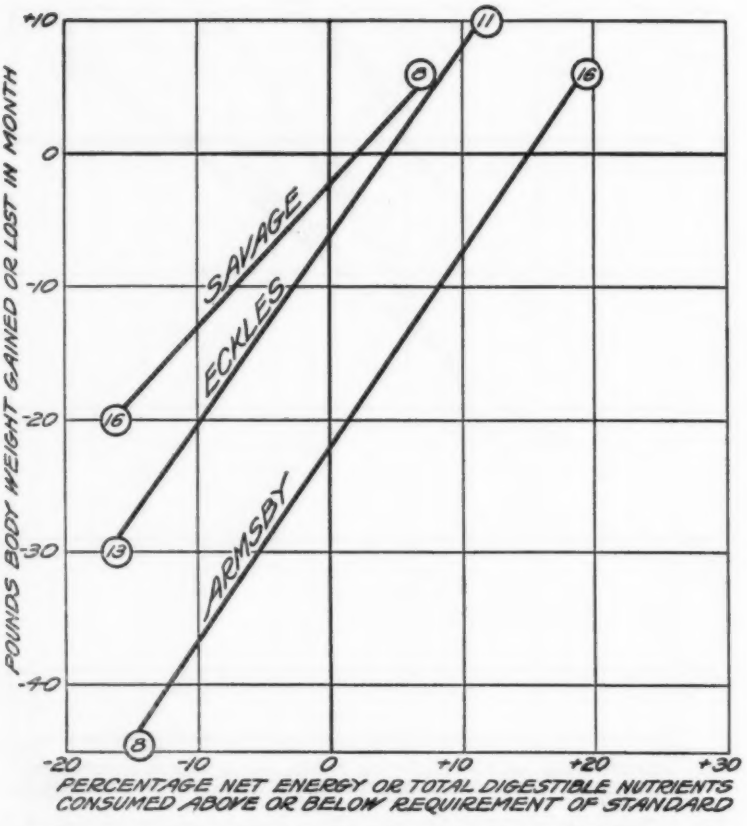

FIG. 2. RELATIONS BETWEEN CHANGE IN BODY WEIGHT AND PER CENT OF NET ENERGY OR DIGESTIBLE NUTRIENTS CONSUMED ABOVE OR BELOW REQUIREMENTS OF ARMSBY, ECKLES AND SAVAGE STANDARDS

Graphs plotted from data in table 2

1 and 2, the percentage of nutritive energy consumed above or below the requirements being used as abscissa, and the number of pounds gained or lost in the month, as ordinate.[6] In the figures, the points representing the change in body weight for each of the given percentages of nutritive energy consumed above or below requirement have been joined by straight lines. The figures inclosed by small circles give the number of cases on which each point in the chart is based. The point where the straight line for each standard crosses the line of zero change in weight may be taken as approximately indicating the percentage of nutritive energy above or below the requirement of each standard which the cows used in these experiments needed to maintain them at uniform body weight. The high-fed cows, according to these results, needed just the quantity of total digestible nutrients which would be allowed by the Savage standard, 8 per cent more net energy than would be allowed by the Eckles standard, and 17 per cent more net energy than would be allowed by the Armsby standard. The less highly fed cows required 2 per cent more than would be allowed by the Savage standard, 4.5 per cent more than would be allowed by the Eckles standard, and 15 per cent more than would be allowed by the Armsby standard. The agreement between the two sets of results seems to us remarkably close, in view of the small number of cases on which some of the points in the charts are based. Several circumstances indicate, however, that the number of cases on which these points are based is really sometimes too small to give entirely satisfactory results. Among these, the widely different slants for the lines for the various standards are particularly noticeable. In figure 3, the two sets of results have been combined, and present a

[6] The data given in tables 1 and 2 may, of course, be handled in several other ways. For instance, the 15 or 20 cows which, on the average, maintained uniform body weight on rations near the requirement of each of the standards may be selected, and their average percentage consumption above or below the requirement of each standard may be calculated. The writer has tried several different ways of handling the data and found that they all lead to the conclusion that cows maintain uniform body weight when fed approximately according to the Eckles or Savage standard, and lose weight fairly rapidly, when fed according to the Armsby standard.

more convincing appearance. According to the results presented in this figure, the cows from which these records were obtained would have been kept at uniform body weight by a ration just

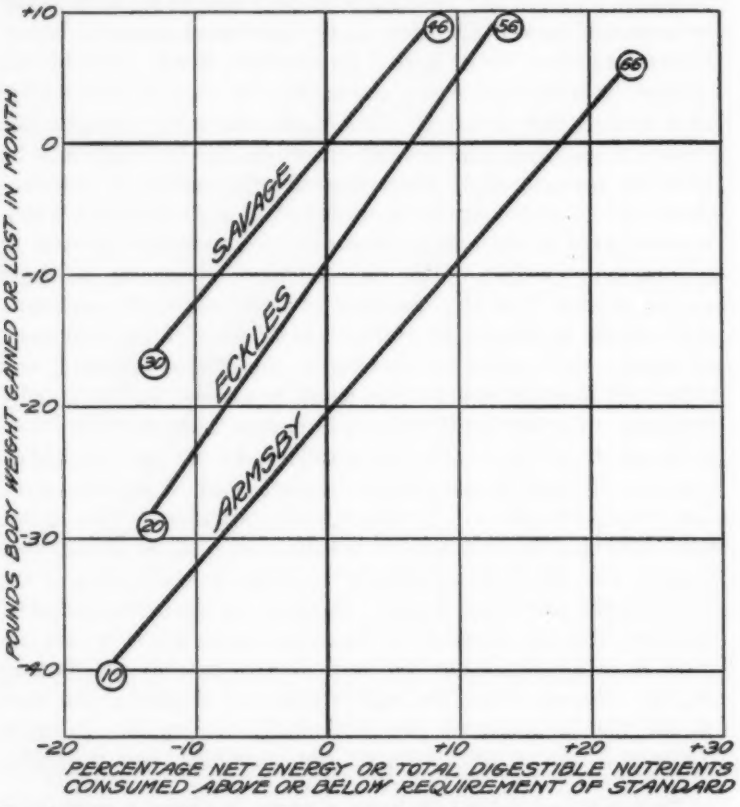

FIG. 3. RELATIONS BETWEEN CHANGE IN BODY WEIGHT AND PERCENTAGE OF NET ENERGY OR TOTAL DIGESTIBLE NUTRIENTS CONSUMED ABOVE OR BELOW REQUIREMENTS OF ARMSBY, ECKLES AND SAVAGE STANDARDS

Graphs plotted from combined data of tables 1 and 2

equal to that allowed by the Savage standard, 6.5 per cent more than that allowed by the Eckles standard, and 18 per cent more than that allowed by the Armsby standard.

Eckles' results and ours taken together point strongly to the conclusion that milking cows fed according to the Armsby standard would receive considerably less nutritive energy than was necessary to keep them at uniform body weight; for reasons which have already been given, the results point also to the conclusion that they would not receive enough to keep them in energy equilibrium. Just why the results obtained under practical conditions should disagree with the experiment of Kellner on which Armsby's standard is based can not be fully decided from the evidence at present in existence. It seems to us not unlikely, however, either that the cow used by Kellner may have secreted milk with more than average economy, or that there may have been some error in interpreting the results. In any case, it is clear that the extensive results obtained under practical conditions are the safer guide for the feeding of cows in practice.

INDICATIONS REGARDING THE RELATIVE PRACTICAL USEFULNESS OF NET ENERGY VALUES AND TOTAL DIGESTIBLE NUTRIENTS TO BE GAINED FROM THE RESULTS SO FAR REPORTED

The quantities of net energy and of total digestible nutrients consumed by the cows used in Eckles's experiments have been calculated and compared with their requirements according to the Eckles and Savage standards respectively.[7] These cows consumed, on the average, 1.7 per cent more net energy than they required according to the Eckles standard, and 2.1 per cent less total digestible nutrients than they required according to the Savage standard.

Eckles's results and ours agree more closely, therefore, in regard to the total digestible nutrients necessary to maintain uniform body weight than in regard to the net energy. Whereas his cows required 2.1 per cent, less total digestible nutrients than ours to maintain uniform body weight, they required 6.5 minus 1.7, or 4.8 per cent less net energy. As the quantities of total

[7] In calculating the total digestible nutrients and the net energy contained in the foods consumed by Eckles's cows, we have used the tables of Henry and Morrison (6) and of Armsby (1), respectively. See above.

digestible nutrients in different feeding stuffs do not run parallel to their net energy values, it becomes of interest to inquire into the respective proportions of total digestible nutrients and of net energy in Eckles's feeds and in ours. The results furnish a preliminary test as to whether total digestible nutrients or net energy values are a better measure of the relative values of different feeds for maintaining uniform body weight.

The total feed consumed by Eckles's cows contained 42,044 therms net energy and 48,822 pounds total digestible nutrients; the ratio between therms net energy and pounds total digestible nutrients in his feeds was therefore $\frac{42{,}044}{48{,}882}$ or 0.861. Similarly, our cows consumed 48,140 therms net energy and 53,227 pounds total digestible nutrients, and the corresponding ratio in the case of our cows was 0.904. For every hundred pounds of total digestible nutrients, therefore, Eckles's cows consumed 86.1 therms net energy, while our cows consumed 90.4 therms net energy, or about 5 per cent more than his. It will be recollected that our cows had to consume only 2.1 per cent more total digestible nutrients to keep them at uniform body weight than Eckles's cows and 4.8 per cent more net energy. The results, therefore, point to the conclusion that total digestible nutrients are a better index of the relative values of feeds for maintaining uniform body weight in dairy cows than are net energy values. While the two sets of results are only 2.1 per cent apart when the total digestible nutrient content of the two sets of feeds are considered, they are 4.8 per cent apart when the net energy values are considered; and this latter discrepancy is fairly satisfactorily explained from a consideration of the respective quantities of total digestible nutrients and of net energy contained in the two sets of rations.

There is further reason to think that the quantity of total digestible nutrients necessary to keep dairy cows at uniform body weight is surprisingly constant when it is determined, as in Eckles's experiments and ours, for a number of cows over considerable periods and calculated according to the requirements of the Savage standard.

Haecker, like Eckles, carried out experiments in which

the quantities of feed necessary to keep milking cows at uniform body weight were determined. Haecker's experiments differed from Eckles's, however, in that his cows were not kept farrow, and that some of them were not mature. As the cows used by Haecker were not kept on the experiments for as long as 200 days consecutively, it is probable that some of them would have shown a tendency to build muscle tissue at the expense of body fat, and might, therefore, have gained weight to some extent on a barely sufficient ration, or maintained uniform weight on a somewhat deficient ration.

In spite of these possibilities, we have thought it worth while to compare the total digestible nutrients[8] consumed by Haecker's cows with their requirements for maintenance and milk yield according to the Savage standard. The results show that these cows had to consume, on the average, 5 per cent less total digestible nutrients than the Savage standard calls for in order to maintain uniform body weight.

Haecker's cows required somewhat less digestible nutrients to keep them at uniform body weight than did either Eckles's or ours, as was to have been expected from the fact that they were not always either mature or farrow. It will be remembered that Eckles's cows required 2.1 per cent less than the Savage standard; and ours, just the requirement of the Savage standard. The greatest difference, therefore, lies between our results and Haecker's, and amounts to 5 per cent.

[8] In estimating the total digestible nutrients consumed by Haecker's cows, we have used the figures which he obtained from his own analyses of the feeds used by him. As far as it was possible, however, from the data given by Haecker, we have calculated the quantities of total digestible nutrients in the food consumed by his cows according to the figures given by Henry and Morrison in the 1921 edition of "Feeds and Feeding" (6). In most cases there is a very close agreement between these results and those obtained by Haecker from his own analyses; where a difference exists, it has been of such a nature as to indicate that the agreement between Haecker's results, on the one hand, and those of Eckles and ourselves, on the other, in regard to the quantity of total digestible nutrients necessary to maintain uniform body weight in milking cows would be closer if the total digestible nutrients in Haecker's feeds were calculated according to the figures of Henry and Morrison than it is when they are calculated from the results of his own analyses. See (5).

This remarkable agreement between three independent sets of results carried out under different climatic conditions, with different kinds of cows, and with different kinds of feed, shows that the figures given by Henry and Morrison for total digestible nutrients furnish a system for estimating the relative usefulness of feeds as sources of nutritive energy which is, to say the least, very fairly accurate. And, as the disagreement between our results and Eckles's in terms of net energy value is larger than in terms of total digestible nutrients it is proper to inquire whether the latter system is not just as good or better for practical purposes than the former. It is, of course, fully realized that the considerations which have just been given are not sufficient to answer finally so important a question as this. Experiments to throw further light on it are now being planned at Beltsville, and the results will be published as soon as possible after they have been completed. In the meantime, however, it is proposed to inquire critically into the physiological considerations underlying the use of the two sets of units, and into the experimental basis for the figures which exist at present in the literature. This will be done in the next article of this series.

SUMMARY

Recent results obtained at the Beltsville experiment farm are in close agreement with the extensive investigations of Haecker in regard to the quantities of total digestible nutrients required to keep milking cows at uniform body weight. When the results obtained by Eckles in the same field are calculated in terms of total digestible nutrients, they also are found to be in close agreement with the two sets of results above mentioned.

The results obtained by Eckles do not agree with Armsby's feeding standard in regard to the net energy requirements of milking cows. Our own results are decidedly more nearly in agreement with Eckles on this question than with Armsby. A study of the Armsby standard shows that it is not based on any experiments carried out under conditions approaching those which obtain in practice, but on a train of physiological reasoning which rests on an experiment of Kellner in which the complete

energy balance was obtained in one milking cow for a period of two weeks. Although two other cows were considered, both were somewhat less economical, and were not used in the calculations.

The agreement between Eckles's results and ours is closer in terms of total digestible nutrients than in terms of net energy. It is proper, therefore, to make a critical examination into the physiological considerations underlying the determination of net energy values and into the experimental basis for the net energy figures which exist at present in the literature.

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A COMPARATIVE STUDY OF METHODS FOR DETERMINING THE PER CENT OF BUTTERFAT IN DAIRY PRODUCTS*

II. ICE CREAM

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The first paper (1) on this subject deals with a comparison of the Mojonnier, the Modified Babcock and the Troy-Fucoma method for determining the per cent of butterfat in ice cream. Observation of the Troy-Fucoma method in these first trials frequently showed a hazy film in the fat column. This film usually disappeared when samples were centrifuged in a heated tester and run for a few minutes longer than the prescribed four minutes. It was also observed that results obtained tended to be higher when the centrifuging period was extended beyond four minutes.

Our next efforts therefore have been confined to a study as to the influence of the following factors:

1. Influence of the length of centrifuging on the per cent of fat and the appearance of the fat column.
2. Influence of temperature of tester and fat column on appearance of the fat column and the final per cent of fat.
3. Accuracy of the Torsion Balance for weighing the charge.

INFLUENCE OF LENGTH OF CENTRIFUGING AND HEATED TESTER

The percentage of fat as obtained by duplicate determinations on the Mojonnier tester were used as a standard of comparison in each instance. Several duplicate samples of ice cream were prepared according to Troy-Fucoma directions, weighing being done with a Torsion cream balance into previously calibrated

* Received for publication March 1, 1925.

test bottles.[1] These samples were then centrifuged for different periods of time. The per cent of fat and appearance of the fat column was noted in each case and especially the presence or absence of the characteristic hazy film at the bottom of the fat column. Results are reported in table 1. A study of the data in

TABLE 1

Influence of length of time of centrifuging on the per cent of fat

MOJONNIER TEST	PER CENT OF FAT OBTAINED WHEN CENTRIFUGED FOR			INCREASE IN PER CENT OF FAT OVER FIRST FUCOMA READING	
	Four minutes*	2 × two minutes†	3 × two minutes‡	2 × two minutes	3 × two minutes
C. A. C. ice cream mix, 10/4/24 13.64 to 13.71, average 13.675	13.6	13.7	13.9	0.1	0.3
	13.8	13.8	14.0	0.0	0.2
	13.4	13.7	13.9	0.3	0.5
	13.8	13.8	14.0	0.2	0.2
	13.0	13.4	13.8	0.4	0.8
	13.9	13.9	14.2	0.0	0.3
	13.6	13.7	13.9	0.1	0.7
	13.2	13.6	13.9	0.4	0.7
	13.0	13.4	13.8	0.4	0.8
	13.0	13.2	13.6	0.2	0.6
	13.2	13.4	13.7	0.2	0.5
	Film prominent	Film prominent	Film inconspicuous		
	VARIATION FROM AVERAGE MOJONNIER TEST			VARIATIONS FROM FIRST FUCOMA READINGS	
	−0.166%	−0.015%	+0.206%	0.2%	0.5%

* Four minutes = bringing tester up to speed and allowing to run at full speed for four minutes.

† Two × two minutes = bringing tester up to speed, running for two minutes with steam off, then bringing up to speed again and running for two minutes with steam off.

‡ Three × two minutes = bringing tester up to speed three times and allowing it to run for two minutes each time with steam off.

table 1 seems to indicate that centrifuging for four minutes or 2 times two minutes is not a sufficient length of time to remove the hazy film or to bring all of the fat up into the fat column. Thus after four minutes centrifuging, there was a pronounced film and the results in the majority of the instances were lower than the Mojonnier.

[1] Calibration of Fucoma test bottles was made possible through the courtesy of Dr. E. M. Bailey, of the Connecticut Agricultural Experiment Station, New Haven, Conn.

In three of the samples the Fucoma was 0.675 per cent lower than the Mojonnier. Bringing the centrifuge up to speed again at the end of two minutes failed to eliminate the film but gave results which compared closely with the Mojonnier method. However, it is interesting to note that bringing the centrifuge up to speed a third time still further raised the test and almost eliminated the film. In every instance except one, the results after bringing the centrifuge up to speed a third time, were higher than the Mojonnier tests. The average of eleven tests of the same sample gave results 0.2 per cent higher than the Mojonnier. The importance and influence of length of centrifuging is also brought out in the summary of the two last columns in table 1. Thus bringing the centrifuge up to speed a second time at the end of two minutes raised the test 0.2 per cent, while a third bringing up to speed increased the test 0.5 per cent over centrifuging it for only four minutes.

In order to determine the maximum time required for centrifuging and to study the influence of a heated tester, a second series of samples were prepared. The steam turbine centrifuge was warmed up by turning on steam and allowing it to run idle for about ten minutes. This brought the centrifuge to a temperature of about 135° to 140°F. The samples were then placed in the heated tester and centrifuged for seven minutes with steam turned on during the entire time. After noting the nature of the fat column and the per cent of fat, the samples were centrifuged for two more minutes and read again. As is indicated in table 2, all fat columns were distinctly clear with no film. Whether the elimination of the film was due to the heated tester or centrifuging at constant speed for seven minutes is a question, but as there was still a slight film when the samples were centrifuged for 3 times two minutes, it seems reasonable to conclude that a heated tester aids materially in removing the film. The readings after seven minutes centrifuging checked closely with the Mojonnier, but an additional centrifuging of two minutes caused a higher reading in 4 out of 7 samples. Since results in table 2 indicated that centrifuging for seven minutes in a heated tester gave results which not only checked closely with the

Mojonnier, but fat columns which were free from the hazy film, we tried lowering the centrifuging time to six minutes with constant pressure and speed, and with steam turned on during the entire six minutes. Results reported in table 3 show that while the tests compare closely with the Mojonnier, six minutes centrifuging was not sufficient to entirely eliminate the hazy film in all of the fat columns. Therefore, after reading the samples, they were again placed in the centrifuge and whirled for another

TABLE 2

Influence of heating the tester and increasing the length of time of centrifuging on the clearness of fat column and final per cent of fat

MOJONNIER TEST	FUCOMA TEST RUN FOR			INCREASE IN PER CENT OF FAT OVER FIRST FUCOMA READING	
	Seven minutes*	Nine minutes*	Nine minutes water bath†	Nine minutes	Nine minutes plus water bath
C. A. C. ice cream mix, 10/20/24 14.48 to 14.45, average 14.465	14.6	14.7	14.8	0.1	0.2
	14.7	14.9	14.9	0.2	0.2
	14.6	14.6	14.6	0.0	0.0
	14.8	14.8	14.8	0.0	0.0
	14.6	14.8	14.8	0.2	0.2
	14.6	14.6	14.6	0.0	0.0
	14.5 Fat column clear, no film	14.6 Fat column clear, no film	14.6 Fat column clear, no film	0.1	0.1
	AVERAGE VARIATION FROM MOJONNIER TEST			AVERAGE VARIATION FROM FIRST FUCOMA READING	
	+0.163%	+0.248%	+0.265%	0.086%	0.10%

* Read from tester.

† Placed in water bath at 130°F. for three to four minutes.

three minutes. This eliminated the film but also increased the size of the fat column. The data in table 3 further stresses the importance of length of centrifuging, but as shown in all the other tables also brings out that exhaustive centrifuging results in tests which are from 0.2 to 0.4 per cent higher than the Mojonnier. This probably is due to the use of amyl alcohol. A study of its chemical properties leads us to believe that some of the amyl alcohol mixes with the milk fat. The fat column therefore is not pure milk fat, but in reality a mixture of milk fat and amyl alcohol.

However, it is also true that by carefully controlling the period of the centrifuging and the speed of the tester, results may be obtained which check closely with the Mojonnier. Thus when centrifuged for six minutes, 7 samples out of 10 checked within 0.15 per cent while the highest individual variation was 0.265 per cent. When centrifuged for seven minutes, 5 samples out of 7 checked within 0.15 per cent and the largest individual variation was 0.345 per cent. When it is recalled that considerable varia-

TABLE 3

Showing influence of centrifuging for six minutes with and without use of water bath

MOJONNIER TEST	FUCOMA TEST RUN FOR			INCREASE OF PER CENT OF FAT OVER FIRST FUCOMA READING	
	Six minutes*	From water bath†	Nine minutes‡	From water bath	Nine minutes
C. A. C. ice cream mix, 10/20/24 14.48 to 14.45, average 14.465	14.7	14.6	14.8	−0.1	+0.1
	14.6	14.6	14.7	+0.0	+0.1
	14.2	14.3	14.6	+0.1	+0.4
	14.2	14.2	14.4	+0.1	+0.3
	14.6	14.7	14.75	+0.1	+0.15
	14.3	14.4	14.6	+0.1	+0.3
	14.5	14.6	14.8	+0.1	+0.3
	14.6	14.7	14.8	+0.1	+0.2
	14.4	14.5	14.7	+0.1	+0.3
	14.6	14.7	14.7	+0.1	+0.1
	Film prominent	Slight film	No film		
	VARIATION FROM AVERAGE MOJONNIER TEST			VARIATION FROM FIRST FUCOMA READING	
	+0.005%	+0.065%	+0.215%	0.090%	0.225%

tions are encountered even on the Mojonnier tester, these results are surprisingly close on a product like ice cream.

In view of these results the authors suggest the following modifications from the Troy-Fucoma method:

1. Weigh out and prepare samples as per directions of the Fucoma Co., except that 6 cc. of water are used instead of 5 cc.

2. Place samples in heated centrifuge and bring the tester up to speed.

3. *Centrifuge for a period of not less than six minutes nor more than seven minutes at a constant and full speed.* This is perhaps best done in a steam turbine tester by regulating the steam pres-

TABLE 4

Results of modified Fucoma method as compared with the Mojonnier method for determining the per cent of the fat in ice cream

ICE CREAM	MOJONNIER TEST	FUCOMA TEST	VARIATION FROM MOJONNIER
Chocolate	9.46	9.6	+0.14
Chocolate	9.48	9.65	+0.17
Chocolate	12.26	12.30	+0.04
Chocolate	12.19	12.10	−0.09
Strawberry	7.98	8.10	+0.12
Strawberry	7.95	8.05	+0.10
Vanilla	9.87	9.80	−0.07
Vanilla	9.69	9.80	+0.11
Vanilla	11.62	11.70	+0.08
Vanilla	11.61	11.60	+0.01
Vanilla	12.89	13.00	+0.11
Vanilla		14.10	−0.11
		14.10	−0.11
		14.10	−0.11
		14.05	−0.16
		14.20	−0.01
		14.10	−0.11
		14.15	−0.06
		14.20	−0.01
		14.10	−0.11
		14.20	−0.01
		14.15	−0.06
		14.00	−0.21
		14.30	+0.09
		14.40	+0.19
		14.00	−0.21
		14.20	−0.01
		14.40	+0.19
		14.30	+0.09
		14.40	+0.19
		14.30	+0.09
		8.70	−0.08
		8.80	+0.02
		8.80	+0.02
		8.70	−0.08
		8.75	−0.03
		8.80	+0.02
		8.75	−0.03
		8.90	+0.12
		9.00	+0.22
		8.90	+0.12
		9.00	+0.22
		9.00	+0.22
		9.00	+0.22
		9.00	+0.22

C. A. C. vanilla ice cream mix — 14.20 to 14.22, average 14.21

Chocolate ice cream mix — 8.775 to 8.793, average 8.78

All but 16 of the Fucoma tests reported in table 4 were conducted by George Brigham as partial fulfillment of the requirements of the course in Advanced Dairying.

sure so as to give the necessary revolutions per minute (1000 to 1200 revolutions per minute, depending on the diameter). The steam should be on during the entire run so as to maintain constant speed and also to maintain the heat in the tester. If no provision is made for heating the tester, it is a good plan to warm up the tester by allowing it to run empty with steam turned on for about 5 minutes just previous to centrifuging the samples.

4. After whirling the samples in a heated centrifuge for six to seven minutes, remove them from tester and place in a hot water bath at 130°F. for five minutes. Obviously the level of water should be such that the fat columns are immersed.

5. Read each sample directly from water bath.

Results on Fucoma tests made according to the suggested modifications are reported in table 4. This includes not only 25 tests of vanilla ice cream but 18 of chocolate and 2 of strawberry fruit ice cream.

A study of the data in table 4 shows that when the suggested modifications of Fucoma tests were followed:

22 samples or 48.88% of samples checked within 0.1% from Mojonnier
33 samples or 73.33% of samples checked within 0.15% from Mojonnier
38 samples or 84.44% of samples checked within 0.20% from Mojonnier
45 samples or 100.00% of samples checked within 0.25% from Mojonnier

These results check much closer with the Mojonnier than those reported in our first paper (1). This we believe is due to the careful standardization of: (1) the time and speed of centrifuging; (2) the temperature of centrifuging; (3) the temperature at which the fat columns were read.

INFLUENCE OF TEMPERATURE OF FAT COLUMNS WHEN READ

There was some question as to the influence of the temperature of the water bath on the per cent of fat. To check this factor and to determine which temperature gave the closest comparable results with the Mojonnier, a series of tests were made in which samples were first read at a temperature of 140° to 135°F. and subsequently at 130° to 125°F. Results are reported in table 5. Practically all of the tests read at 140° to 135°F. gave results averaging about 0.1 per cent higher than those at 130° to 125°F.

As the results obtained at 130° to 125°F. checked closer with the Mojonnier, this temperature is suggested in preference to the higher temperature.

ACCURACY OF THE TORSION BALANCE FOR WEIGHING THE CHARGE

A series of trials were run comparing the Torsion balance with the analytical balance. For this purpose a wire holder was devised for use in weighing charges with the Fucoma test bottles on the analytical balance. For comparison 24 tests were weighed out on the analytical balance and 24 on the Torsion cream

TABLE 5

Influence of varying temperature of the water bath on the per cent of fat obtained

MOJONNIER TEST	FUCOMA TESTS READ AT		PER CENT OF VARIATION FROM SECOND OVER FIRST READING	VARIATION FROM MOJONNIER	
	130 to 125°F.	140 to 135°F.		130 to 125°F.	140 to 135°F.
C. A. C. ice cream mix, 10/4/24 13.64 to 13.67, average 13.675	13.7	13.7	+0.0	0.025	0.025
	13.8	13.9	+0.1	0.125	0.225
	14.1	14.2	+0.1	0.425	0.525
	13.9	13.9	+0.0	0.225	0.225
	13.6	13.7	+0.1	0.075	0.025
	13.8	13.9	+0.1	0.125	0.225
	13.8	14.0	+0.2	0.125	0.325
	13.6	13.6	+0.0	0.075	0.075
	13.9	14.0	+0.1	0.225	0.325
	13.7	13.8	+0.1	0.025	0.125
	13.7	13.8	+0.1	0.025	0.125
	13.8	13.9	+0.1	0.125	0.225
Average variation.....			0.083%	0.125%	0.204%

balance. These total of 48 tests were then prepared and centrifuged in exactly the same manner. After centrifuging, all tests were read from a water bath at 130°F. Results are given in table 6. Judging from results in table 6, the Torsion balance is sufficiently accurate and compares favorably with the chemical balance.

SUMMARY AND CONCLUSION

1. Further studies were made with the Troy-Fucoma method for determining the per cent of fat in ice cream. The object

was to determine the influence of (*a*) length of time of centrifuging (*b*) heating the centrifuge (*c*) and the temperature of the fat column when read. The Mojonnier method was used as a standard of comparison.

2. The accuracy of the Torsion cream balance for weighing the 5-gram charges used in the Fucoma method was determined by

TABLE 6

Accuracy of Torsion balance when used for weighing 5-gram charge

MOJONNIER TEST	FUCOMA TEST		VARIATION OF FUCOMA TESTS FROM MOJONNIER	
	Cream balance	Analytical balance	Cream balance	Analytical balance
C. A. C. ice cream mix, 10/4/24 13.64 to 13.71, average 13.675	13.4	13.6	−0.275	−0.075
	13.8	13.9	+0.125	+0.225
	13.9	14.1	+0.225	+0.425
	13.7	13.9	+0.025	+0.225
	13.2	14.0	−0.475	+0.325
	13.6	13.8	−0.075	+0.125
	13.7	13.8	+0.025	+0.125
	13.4	14.0	−0.275	+0.325
	13.8	14.1	+0.125	+0.425
	13.4	14.0	−0.275	+0.325
	13.4	14.0	−0.275	+0.325
	13.7	13.9	+0.025	+0.225
	13.7	13.9	+0.025	+0.225
	13.9	13.9	+0.225	+0.225
	14.2	14.2	+0.525	+0.545
	13.9	13.9	+0.225	+0.225
	13.7	14.1	+0.025	+0.425
	13.9	13.9	+0.225	+0.225
	14.0	14.0	+0.325	+0.325
	13.6	13.8	−0.075	+0.125
	14.0	14.0	+0.325	+0.325
	13.8	14.0	+0.125	+0.325
	13.8	13.9	+0.125	+0.225
	13.9	14.0	+0.225	+0.325
Average variation			0.193%	0.277%

comparing results obtained with a Torsion balance with results when charges were weighed on an analytical balance.

3. Centrifuging the ice cream samples for four minutes or 2 times two minutes in the Fucoma centrifuge gave variable results which in the majority of instances were lower than the

Mojonnier test. Fat columns frequently had a hazy film at the bottom of the fat column.

4. Centrifuging 3 times two minutes or six to seven minutes with constant speed gave results which checked closely with the Mojonnier test and fat columns which were clear and distinct.

5. Centrifuging for nine minutes with constant speed gave readings which were from 0.2 to 0.4 per cent higher than the Mojonnier test. High results are probably due to part of the amyl alcohol mixing with the milk fat and appearing in the fat column as a mixture of amyl alcohol and milk fat.

6. A heated tester greatly aided in eliminating the hazy film at the bottom of the fat column.

7. Readings compared closer to Mojonnier test when read at 130° to 125° than if a temperature of 140° to 135°F. is used for water bath.

8. A good Torsion balance is sufficiently accurate for use in weighing out charges for the Troy-Fucoma or Babcock method.

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THE DESTRUCTION OF BACTERIA IN THE ROLLER PROCESS OF MILK DRYING*

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For several years past the dried milk prepared in the factories controlled by these laboratories has been subjected to daily bacteriological examination. The results of these examinations have demonstrated the remarkable degree of bacterial purity which can be attained by a careful attention to details of technique, and to the proper control of the conditions under which the machines are run.

Constant inoculation experiments have also demonstrated the complete freedom from tuberculous infection of dried milk prepared at these factoies. This might have been due to one of three causes:

a. Failure of the inoculated animals to react.
b. Freedom from tuberculous infection of the milk received at the source of manufacture.
c. Destruction during the drying process of B. tuberculosis, if present in the milk.

The probability of the first of these causes being responsible for the negative results is extremely remote when the large number of experiments is considered; it becomes even less likely when it is remembered that inoculations, by an identical technique, of animals with milk known to be infected with tuberculosis have produced positive results.

While the milk received at the factories is known to be in general free from tuberculous infection (this has on many occasions been demonstrated by actual inoculation experiments), there is, nevertheless, the possibility, where large volumes of milk are received, of slight tuberculous infection on some occasions.

While, therefore, (*b*) was a possible, if not a probable, cause

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of the results obtained, it seemed desirable to investigate how far the drying process itself could be responsible for destruction of tuberculous infection in the event of its accidentally occurring in the milk supplies.

Moreover, ordinary routine bacteriological examinations are no guide as to the possibility of the survival of the pathogenic types, such as B. typhosus and pathogenic micrococci, which might conceivably be present in the original milk.

Apart from Delépine's work, there have been published, so far as the authors are aware, no experiments which place this matter beyond doubt.

Delépine (1) was concerned chiefly with B. tuberculosis; this was the only specific organism which he tested for viability after undergoing the drying of milk by the roller process. His observations on the survival of other types of bacteria in this process were confined to a general study of the flora of the milk before and after drying, and his conclusions were that while micrococci and non-sporing bacilli were not found in the dried milk as it came from the knives of the machine, sporing bacilli were fairly abundant. Delépine's observations on the bacillus tuberculosis will be considered in a later section of the present paper. Supplee and Ashbaugh (2) showed the extent of the reduction in the total bacterial content brought about by the roller process, and how this may be improved by care in technique at the factories. The same subject has been dealt with by the present authors (3).

None of these investigations, however, demonstrated the effect of the roller process upon various types of bacteria treated separately, and the present series of experiments was undertaken with this object.

SCOPE OF THE INVESTIGATION

The types of bacteria to be investigated were four in number:

a. Non-sporing bacteria, including various types of micrococci
b. Sporing bacilli
c. B. tuberculosis (human)
d. B. tuberculosis (bovine)

Some living tubercle bacilli of bovine origin were found to have survived treatment (by the roller process).

Owing to the fact that a special technique is necessary in investigating B. tuberculosis, this part of the investigation is treated separately.

For the experiments with the other types of bacteria there were selected typical strains which may be taken as representative of the various main types into which bacteria can be grouped. No proteus strain was included, but apart from its strong proteolytic properties this organism is of the same general class as B. coli and B. cloacae, i.e., it is a small motile non-capsulated gram negative organism. It may be pointed out that the various strains tested, though not pathogenic strains, are all representative of pathogenic types, and evidence as to the destruction of B. coli is equally applicable to (for example) B. typhosus. A similar consideration applies to all the other strains tested.

The names of the organisms are set out in the tables, and there is nothing to be said of their characters, except that they were typical in each case.

1. Bacteria, other than B. tuberculosis

Experimental method. Half a gallon of ordinary full cream milk was sterilized by keeping it just below 100°C. for one hour. A fresh agar slope culture of the organism to be tested was then emulsified into about 5 cc. of the cold milk and the emulsion added to the bulk when cold, which was allowed to stand overnight at room temperature.

A count of the bacteria in the milk before drying was made on agar plates kept for forty-eight hours at 37°C.

It is obviously undesirable that the drying of inoculated milk should be carried out in a factory and on machines engaged upon the production of dried milk for infant feeding. These experiments were, therefore, carried out upon the laboratory experimental drying plant. It may, however, be emphasized that

this experimental drying plant is identical in every respect with the factory machines, with the exception that the length of the rollers is considerably reduced. The conditions maintained during these experiments, i.e., temperature of solutions, steam pressure, rate of flow of milk, etc., were identical with those ruling at all the factories; these conditions are determined by the laboratory and for them one of the present authors, who is thoroughly familiar with the factory conditions, is in part responsible. The milk receptacles, tanks and feeding pipes of the machine were sterilized before each experiment by means of live steam, and the walls of the drying room, which are lined with white tiles, were kept moist during the whole of the experiment.

The film of dried milk coming away from the knives of the machine was directed by means of sterile spatulae into sterile tins, the lids of which were replaced as soon as all the powder from each drying had been collected.

The dried milk was reconstituted by adding 7 parts of sterile water to 1 part of the powder, warming to 45°C. for ten minutes, and shaking well; 0.5 cc. of the reconstituted milk was then plated to nutrient agar in the usual manner. The plates were incubated for forty-eight hours at 37°C. before examination.

Observations. It will be seen from a study of table 1, that virtually a total destruction of the non-sporing types of bacteria took place. In only one case were there any survivors, and in that case the number of bacteria in the original milk was enormously greater than in any of the others. Moreover, a survival of four bacteria out of a total of 2,000,000,000 is quite negligible.

All pathogenic types likely to be met with in milk are non-sporers, and it is clear from the experiments cited that all of these will be totally destroyed in milk which is dried by the roller process. One organism, of unknown species, was a capsulated organism, having a well marked fat envelope. It might have been expected that a proportion of these would survive, but such was not the case, although as many as 320,000,000 per cc. were found in the milk before drying.

Turning now to table 2, it will be seen that two typical sporing

saprophytes were tested, and that both showed a certain percentage of survivors after drying. This is to be expected, since the resistance of spores to heat is often remarkably great.

The survival of a certain number of sporing types is of no importance from the hygienic point of view, since no sporing strains which would be likely to be found in milk are pathogenic when administered orally. Nevertheless, the presence or absence of sporing bacteria in dried milk gives a valuable indication as to the purity or otherwise of the original milk used, especially as B. welchii (B. enteritidis sporogenes) is a particularly resistant spore-bearer of intestinal origin, whose value as an indicator of excretal contamination is well known. Moreover the presence of large numbers of ordinary aerobic sporing bacilli, which are readily detected by the characteristic appearance of their colonies on agar, would suggest that the general bacterial content of the original milk was high.

TABLE 1

Non-sporing bacteria

LABORATORY NUMBER OF STRAIN	TYPE	NUMBER OF BACTERIA IN ORIGINAL MILK PER CUBIC CENTIMETER	NUMBER OF BACTERIA IN RECONSTITUTED DRIED MILK PER CUBIC CENTIMETER
A. 35	B. coli	300,000,000	0
A. 6	B. cloacoe	2,000,000,000	4
A. 57	Capsulated, produces red pigment	320,000,000	0
A. 94	Sarcina lutea	1,000,000	0
A. 95	Staphylococcus albus	510,000,000	0
A. 97	Streptococcus long chains	10,000,000	0

TABLE 2

Sporing bacteria

LABORATORY NUMBER OF STRAIN	TYPE	NUMBER OF BACTERIA IN ORIGINAL MILK PER CUBIC CENTIMETER	NUMBER OF BACTERIA IN RECONSTITUTED DRIED MILK PER CUBIC CENTIMETER
A. 11	B. mycoides	120,000,000	47,000
A. 96	B. subtilis	2,000,000	50,000

2. Bacillus tuberculosis

Whether or no B. tuberculosis survives the roller process of milk drying is a matter of very great importance, in view of the great use which is now made of dried milk for infant feeding, and of the claim of the manufacturers that by its use all risk of infection by this organism is avoided.

In view of the results of Delépine's investigations (loc. cit.) it seemed advisable to confirm by direct experiments the negative results obtained from routine inoculations in these laboratories, and this section of the present investigation is the outcome.

Review of Delépine's investigation. Delépine in his experiments used 10 gallons of milk which contained 240 cc. of fresh tuberculous milk from a cow which had been under observation for about a month and was giving highly infected milk from three out of four quarters of the udder. In addition to this the 10 gallons of milk contained 250 cc. of milk cultures of the bovine tubercle bacillus, and 5 potato cultures of the same bacillus. Delépine demonstrated that each drop of the final mixture used in each experiment contained tubercle bacilli easily demonstrable. He considered that the number and virulence of the bacilli present in the treated milk were not in excess of what he had observed in the case of naturally infected milk.

He inoculated four guinea pigs with the original milk, two with milk from the churn just before the beginning of the experiment, and two with milk from the tank towards the end of the operations. Four guinea pigs were also inoculated with reconstituted milk taken (*a*) from one roller at the beginning of the operation, (*b*) from the other roller at the beginning of the operation, (*c*) from one roller towards the end of the operation, (*d*) from the other roller towards the end of the operation.

The results of the inoculation experiments are set out in great detail in Delépine's report, but it will only be necessary here to quote a portion of his summary:

> . . . . The tubercle bacilli which had survived . . . . were still capable of producing progressive tuberculosis in guinea pigs inoculated subcutaneously with milk containing these bacilli, but the

course of the disease produced by these bacilli was very much slower than that of the disease produced in guinea-pigs inoculated with the same amount of untreated tuberculous milk. The tuberculosis porduced by the heated bacilli was latent or occult for some four weeks.

Young rabbits fed with milk containing these modified bacilli did not contract tuberculosis.

METHOD ADOPTED IN THE PRESENT INVESTIGATION

One gallon of fresh milk was taken, but not sterilized, since the presence of other bacteria was of no moment. This gallon was divided into two portions, one of which was inoculated with a strain of human B. tuberculosis, and the other with a strain of bovine B. tuberculosis, both strains having been obtained from the National Collection of Type Cultures, Lister Institute. The cultures were both grown on blood serum slopes. To inoculate the milk, about 5 cc. was added to the blood serum culture, and the growth completely scraped off into the milk. The 5 cc. of milk containing the tubercle bacilli was then added to the bulk of the milk in each case, and thoroughly mixed by stirring and shaking.

Fifty cubic centimeters of the milk were centrifugalized at 3000 r.p.m. for ten-minutes and the deposit mixed with 20 cc. of sterile water. Five cubic centimeters of this mixture were then injected into the left thigh of each of two guinea pigs with the usual aseptic precautions. Two guinea pigs were inoculated with the milk containing bovine tubercle bacilli, and two with that containing human tubercle bacilli.

The inoculated milks were dried on the machine already referred to, and under the same conditions as the previous experiments.

The dried powder was reconstituted with sterile water (1 in 8) and two guinea pigs were inoculated with the deposit from the sample which had contained human tubercle bacilli before drying, and two with that which had contained bovine tubercle bacilli.

The results of the inoculation experiments are given in table 3.

The presence of B. tuberculosis in the affected glands and

organs was demonstrated by the Ziehl-Nielsen method of acid fast staining.

OBSERVATIONS

The control animals show that both samples of milk were heavily infected with B. tuberculosis of human and bovine origin, respectively.

TABLE 3

LABORATORY NUMBER	INOCULATED WITH	NUMBER OF WEEKS BEFORE POST MORTEM	RESULT OF POST-MORTEM
107	Milk containing human tuberculosis before drying	8	Tuberculous lesions of superficial and deep inguinal glands, liver and spleen
108	Milk containing human tuberculosis before drying	9	Tuberculous lesions of superficial and deep inguinal, popliteal and retrohepatic glands, and spleen
109	Milk containing bovine tuberculosis before drying	6 (Died)	Tuberculous lesions of popliteal superficial and deep inguinal glands, liver and spleen
110	Milk containing bovine tuberculosis before drying	9	Tuberculous lesions of popliteal superficial and deep inguinal and retrohepatic glands and spleen
111	Milk containing human tuberculosis reconstituted after drying	6 (Paralysis of left hind leg)	No lesions; all glands and organs normal. Well nourished healthy animal, except for paralysis of left hind leg
112	Milk containing human tuberculosis reconstituted after drying	8	Result same as above; perfectly healthy animal
113	Milk containing bovine tuberculosis reconstituted after drying	9	Result same as above
114	Milk containing bovine tuberculosis reconstituted after drying	9	Result same as above

The other four animals which had been inoculated with the milk reconstituted from the dried milk prepared from these

samples showed no lesions, and were all well nourished healthy animals.

The experiments show that the process of drying either killed the bacilli or rendered them avirulent, and confirm the claim of the manufacturers that dried milk prepared as in these experiments is perfectly free from tuberculous infection.

It will be seen that these results are not in agreement with those of Delépine referred to above. It is quite possible that a difference in the conditions of steam pressure and speed of the rollers account for the different results. As in the pasteurization of milk, so also in milk drying it is important that the conditions of heating both as regards time and temperature should be correct, and the present experiments show that under these conditions dried milk prepared from milk infected with B. tuberculosis is not capable of producing tuberculous lesions.

SUMMARY

1. The roller process of milk drying is capable of destroying completely non-sporing bacteria.

2. The spores of spore bearing bacilli are not destroyed in all cases by the process; the number of survivors of this type of organism is probably dependent on the extent to which sporulation has occurred among them.

3. The roller process of milk drying is capable of either destroying or rendering completely avirulent B. tuberculosis of both human and bovine origin.

4. Dried milk adequately prepared by the roller process from highly tuberculous milk is incapable of causing tuberculous lesions in guinea pigs.

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INHERITANCE OF THE PER CENT OF FAT IN A HOLSTEIN HERD*

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Very few results have been reported bearing upon the per cent of butter fat in the milk of the progeny of the female line. Most of the studies on this phase of butter fat inheritance have been developed around the male. The lack of emphasis on the female doubtless results from her relatively small number of progeny.

It is generally known that the milk of cows, even within a breed, varies considerably in the percentage of butter fat it contains. This variation is an individual characteristic. It has been quite definitely shown that each individual cow possesses a certain fairly definite capacity for the production of milk and butter fat, and that the milk, over a number of lactation periods, will contain a fairly constant per cent of fat reaching the correlation coefficient of 0.789 in Holsteins according to Gowen (1).

A study of the records of the Holstein herd at the Connecticut Agricultural College has revealed some interesting and valuable information concerning fat inheritance in two families. The fat percentage of the milk of the descendants of two cows, one testing 3.25 and the other 3.46 per cent, has remained apart to the fifth generation even though bulls were mated in common upon the two families.

REVIEW OF LITERATURE

The mode of inheritance of economic functions in dairy cattle is at present little understood. The literature bearing upon the subject of fat inheritance is indeed scant, and little experimental work bearing directly on this particular problem has been done.

*Received for publication February 6, 1925.

[1] The data were prepared and submitted in partial fulfillment of the requirements for the Master of Science Degree in 1924 by Mr. Burrington.

Recently several investigators have reported the results of studies on the inheritance of milk fat which suggest blended multiple factor inheritance, approaching a little nearer the lower testing parent. Notably among these is the work of Kildee and McCandlish (2), Olsen and Bigger (3), Castle (4), Ellinger (5), and Gowen (6). These results are based upon rather distinct crosses of pure breds, cross breds, and scrubs. Gowen (7) further states from an analysis of the Holstein Advanced Register that the sire exerts an influence upon his daughters (from different dams) expressed by the correlation coefficient of 0.5. Dams on their daughters by different sires have a correlation of 0.413 and he concludes that the sire and dam are about equal in their influence upon the offspring with reference to butter fat test. Furthermore, since the two have a combined correlation of about 0.5, it seems evident that they exert as much influence as all of the other ancestors combined.

COLLECTION OF DATA

Two families of Holsteins have predominated the herd. These two families owed their foundation to two pure bred cows which were purchased in 1903. These cows were Fay M (35970) and Altoana Pietertje A (54656). When purchased, these cows were in calf to DeKol Burke (22991). Shortly after coming to the College herd, they each dropped a heifer calf. Subsequent gestations produced two more heifer calves. The original animals thus laid the foundation for the two families, and each of their daughters were progenitors of a strain within their respective families. Records are now available on five generations of Altoana Pietertje A descendants and four generations of Fay M descendants.

No females, other than the female descendants of these cows, were added to the herd during the greater part of the period under consideration. New blood was introduced into the herd from time to time by the purchase of bulls. These bulls were used indiscriminately on members of both families, although some bulls left daughters in only one family, due to very little use or to the small number of breeding females involved.

Since 1911, samples of milk from each member of the herd have been tested for butter fat at ten-day intervals. Upon the 10th, 20th, and 30th day of each month, samples were taken, both morning and evening, from each member of the herd. The single samples from each animal were composited in proportion to the yield of milk, and tested by the Babcock method. The milk was weighed and recorded after each milking. Earlier than 1911, determinations for fat were made monthly from composite samples. In many instances, the records used are those made under Advanced Registry regulations, being the average for a two-day period for each month.

The data presented here covers a period of twenty years, from 1903 up to and including part of 1923. The five generations of the Altoana Pietertje A family are represented by 16 animals which have completed 48 lactations. The 21 animals composing the four generations of the Fay M family have completed 53 lactations. In compiling the records of these animals, use is made only of lactation periods covering 200 days or more in length. The majority of the lactations cover periods from ten to fourteen months, although a few extend over a longer period. The longest period covers 943 days, while 14 cover less than 300 days. The number of lactations completed by the individual animal varies from one to eight; seven animals have five or more completed lactations, and four have only one.

The herd has been visited from time to time by its quota of disease, accidents, and defectives, although no wholesale disaster has hit it. Infectious abortion and its accompanying ravages have hampered the progress and production of the herd more than any other factor.

PRESENTATION OF THE DATA

The summarized data are presented herewith in graph form.[2] The inherent testing capacity of the animal is determined by dividing the total fat yielded by the total milk.

Figure 1 shows clearly how the two families have tested

[2] The complete data concerning each individual are being published in a Storrs (Conn.) Experiment Station bulletin.

through five and four generations respectively in relation to the breed average and the State legal standard for butter fat.

The average per cent of fat for each generation was determined by taking the direct average of the cows represented. For example, if the first generation contained two animals, one testing 3.59 and the other 3.37, the average is taken to be 3.48 per cent $\left(\frac{3.59 + 3.37}{2} = 3.48\right)$

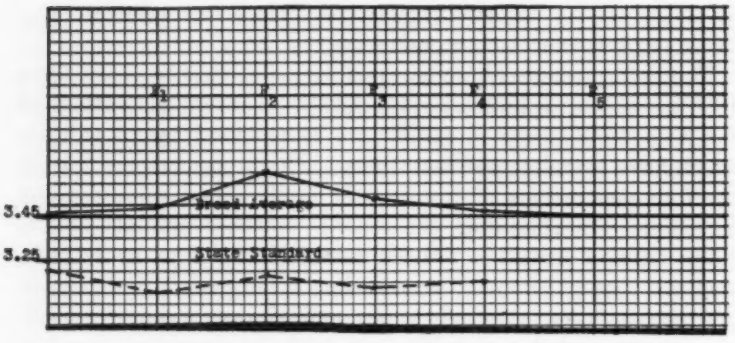

FIG. 1. SHOWING THE PER CENT OF FAT BY FAMILIES AND BY GENERATIONS IN RELATION TO BREED AVERAGE AND LEGAL STANDARD

Solid line above represents the Altoana Pietertje A Family. Dotted line below represents the Fay M Family. Each space represents 0.05.

A study of this graph shows a striking difference in the average tests of these two families. The Altoana Pietertje A family has tested well above the legal standard for fat and has in no case fallen below the breed average of 3.45 per cent. The foundation cow of this family had a test of 3.46 per cent. The F_1, with two animals represented, averaged to test 3.48 per cent; the second generation, consisting of two animals, had an average test of 3.66 per cent; the next generation, composed of five animals, averaged to test 3.51 per cent; the three animals in the fourth generation had an average test of 3.47 per cent; and the fifth generation having only one animal and records available for only a partial lactation tested 3.46 per cent.

The original cow in the Fay M family averaged to test 3.25 per cent. This is 0.21 per cent lower than the foundation cow in the other family. This low testing trait has been passed on through four generations. The first generation progeny composed of two animals have an average test of 3.12 per cent; the

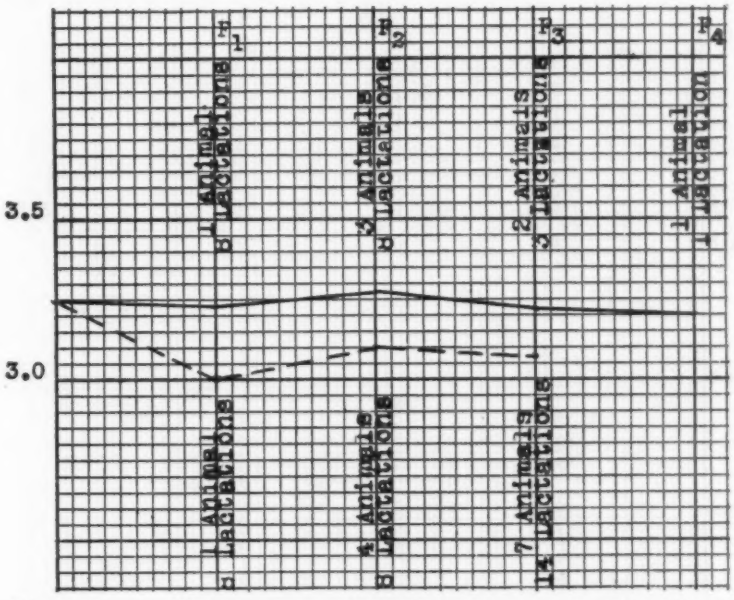

FIG. 2. SHOWING VARIATIONS IN THE PER CENT OF FAT BY STRAINS. FAY M FAMILY

Dotted line represents strain A. Solid line represents strain B. Each space represents 0.05.

second generation has seven animals which tested 3.23 per cent; the nine animals in the third generation have an average test of 3.17 per cent; and the one animal in the fourth generation having records available for only a partial lactation tested 3.2 per cent butter fat.

It will be noted that the last generation represented in the higher testing family averaged to test identically the same as

the original animal. The last generation represented in the other family tested slightly lower than the original animal.

There seems to be no tendency for the tests of these two families to come together. The curve representing the tests of the Altoana Pietertje A family is fairly straight except for the second generation, when it reached the peak with a test of 3.66

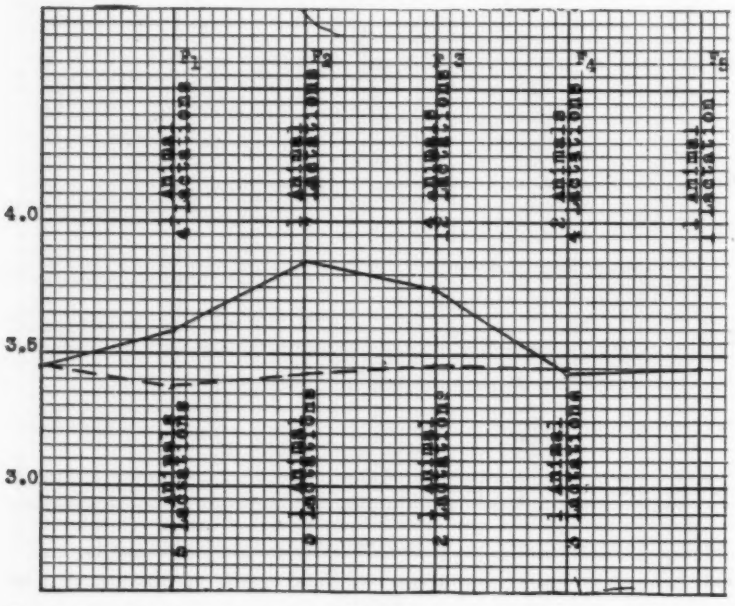

FIG. 3. SHOWING VARIATIONS IN THE PER CENT OF FAT BY STRAINS. (ALTOANA PIETERTJE A FAMILY

Solid line represents strain A. Dotted line represents strain B. Each space represents 0.05.

per cent, which is 0.2 per cent higher than that on either end of the curve. This is due to one very high testing cow, no. 202. The curve for the other family is more nearly a straight line, the original animal testing slightly higher than the average of any other generation of that family.

As shown in figure 2, strain B of the Fay M family has averaged

to test considerably higher than strain A. The two cows in the first generation, those founding the separate strains, show a wider range in the per cent of fat than the animals in any other generation. The higher testing strain presents a much straighter line than does the lower testing group, and there appears to be a tend-

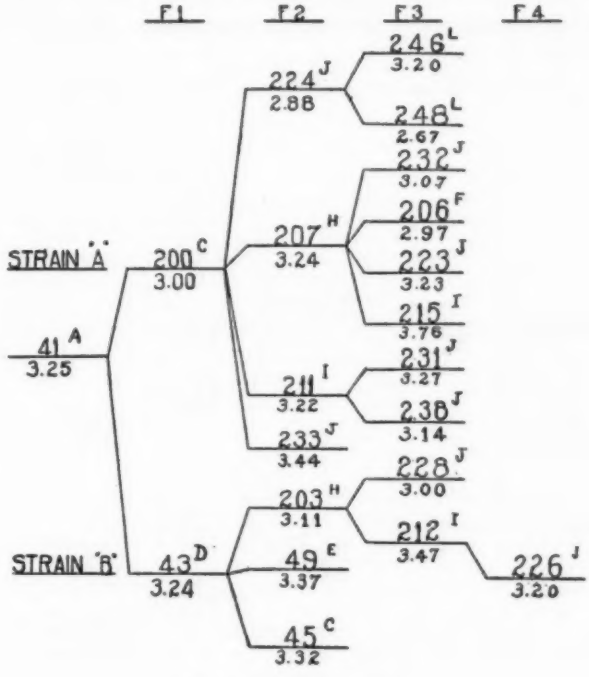

FIG. 4. FAY M FAMILY

ency for these two strains to come together. There is a noticeable decline in the per cent of fat for the higher testing strain and a positive, though not constant, tendency for the lower testing strain to increase in the per cent of fat.

Figure 3 shows that strain A of the Altoana Pietertje A family for the most part has continued to test considerably higher than strain B. The curves representing the two strains of this family

follow somewhat the same trend as those of the Fay M family, although the variations are more pronounced. The extreme variation found in the second generation of the high testing strain can be attributed to the influence of one animal, no. 202, which was an unusually high tester for the breed. The third generation shows a slightly greater variation than the first. The two strains converge in the fourth generation. The per cent of

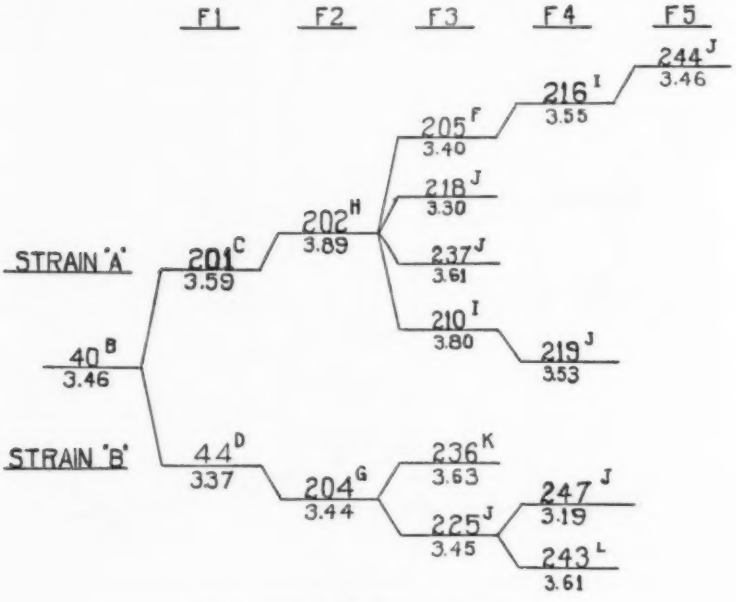

FIG. 5. ALTOANA PIETERTJE A FAMILY

fat of strain A for the fourth and fifth generations has descended to the average of that of the cow which founded these two strains. Records are not available for the fifth generation of the lower testing strain.

Data on the individual animals are shown in more detail in figures 4 and 5 in which the number of the animal is given together with its average fat test. The diagram arrangement

indicates the dam and the progeny of each and the letter identifies the sires.

Interesting facts brought out in these figures are that only two daughters in the low testing family, viz.: Nos. 212 (3.47 per cent) and 215 (3.76 per cent) have reached the original test of the high test family. These two daughters are by a son of a cow in the high test family. Further, there is only one cow in the high test family that has tested below the foundational test of the low test family, namely, no. 247 (3.19 per cent).

TABLE 1

BULLS USED	ALTOONA PIETERTJE A FAMILY (HIGH TEST)							FAT M FAMILY (LOW TEST)							DIFFERENCE IN PER CENT OF FAT IN FAVOR OF HIGH TESTING FAMILY
	High testing strain			Low testing strain				High testing strain			Low testing strain				
	Number of daughters	Number of lactations	Average per cent of fat	Number of daughters	Number of lactations	Average per cent of fat	Average per cent of fat for both strains	Number of daughters	Number of lactations	Average per cent of fat	Number of daughters	Number of lactations	Average per cent of fat	Average per cent of fat for both strains	
C	1	4	3.59				3.59	1	2	3.2	1	8	3.00	3.10	0.53
D				1	5	3.37	3.37	1	1	3.24				3.24	0.13
E*								1	1	3.37				3.37	
F	1	3	3.40				3.40				1	4	2.97	2.97	0.43
G*				1	5	3.44	3.44								
H	1	7	3.89				3.89	1	5	3.32	1	6	3.24	3.28	0.61
I	2	5	3.67				3.67	1	1	3.47	2	4	3.49	3.48	0.19
J	4	9	3.47	1	1	3.19	3.33	2	3	3.10	5	11	3.14	3.12	0.21
K*				1	2	3.45	3.45								
L				1	2	3.66	3.61				2	3	2.93	2.93	0.68

* Bulls K, G and E are of no significance here because each has only one daughter and no comparisons can be made. The records are supplied simply for completeness.

INFLUENCE OF THE BULLS

Table 1 was constructed to determine what influence the several bulls used in the herd may have had on the per cent of fat. A summary of the daughters of each bull is given by strains and by families. Comparisons are made for the daughters of each bull in both strains of each family. A summary of the difference between the families is given in the right hand column.

Naturally variations are found in daughters of the same bull

within the same female line. This variation also has considerable range between the families, varying from 0.68 per cent for bull L (the upper limit) to 0.13 per cent for bull D (the lower limit).

Three of the bulls have daughters in only one strain of one family. However, the seven bulls with daughters in both families give conclusive evidence that the female influence in determining the percentage of fat in the milk of their progeny in this herd was overpowering.

It will be noted that in every case animals by the same sire in the Altoana Pietertje A family have averaged to test above those in the Fay M family. Some bulls that did maintain the average high test in the Altoana Pietertje A family had difficulty in raising the low average of the low testing family, and in a number of cases the test was lowered. This would indicate that there is a tendency for the progeny to resemble the low testing parent. These results are similar to those obtained by Gowen in his study of cross bred daughters of a dairy-beef cross. It further illustrates that the prepotency of the low testing family for low fat tests could not be overcome by the bulls that could maintain an average high test in the other family. Thus it seems that the female may exhibit prepotency for such characters to a high degree.

Reference to bulls used in the herd

A. Count Pietertje Beets 16618
B. Sir Pietertje Lamb 21391
C. Hubbard Netherland DeKol 35221
D. DeKol Burke 22991
E. Crown Prince DeKol Paul 30569
F. Sir Hubbard Pietertje 48557 (sire is C; dam is no. 40)
G. Sir Inka DeKol Lilith 35261
H. Minnie Hark Prince Korndyke 41660
I. Minnie Hark Pietertje Burke 57476 (sire is H; dam is no. 44)
J. King Segis Inka Fayne 97699
K. Segis Fayne DeKol Pietertje 165985 (sire is J; dam is no. 201)
L. King Korndyke Fannie Veeman 199675.

It is unfortunate from the standpoint of such a study that so many different bulls were employed and so few daughters resulted

from their use. It should be remembered, however, that such a study was not contemplated and that the conditions are a natural consequence of maintaining a small herd over a period of twenty years. Some of the bulls did not belong to the College herd.

No attempt has been made to evaluate the fat inherited propensities of the bulls themselves, since short time seven-day tests enter so largely into their pedigree records. However, some of the bulls used are from dams of the high testing family, viz.: Bull F is from the foundation cow 40; bull I is from cow 44, strain B; and bull K is from cow 201, strain A. Table 2 will permit a consideration of such records as are available of these bulls.

In table 2 is given the brief summary of the bulls that were used in the herd that are progeny of certain cows in the high

TABLE 2

Records of bulls bred from herd

BULL	DAM	DAM'S DAUGHTERS	BULLS' OWN DAUGHTERS		BULL'S SIRES' DAUGHTERS	
			High strain	Low strain	High strain	Low strain
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
F	3.46	3.48 (2)	3.40 (1)	2.97 (1)	3.59 (1)	3.05 (2)
I	3.37	3.44 (1)	3.58 (2)	3.48 (3)	3.89 (1)	3.28 (2)
K	3.59	3.89 (1)	3.45 (1)		3.48 (6)	3.19 (7)

testing strain. Because of the relatively few instances they are not dealt with by genetical precision. The test of the dams of these bulls is given as is also their daughters. Furthermore, the tests of the daughters of the sires of these "home bred" bulls is given. The small numbers in parenthesis indicate the number of animals involved in each case.

Bull F is from the foundation cow 40, whose test was 3.46 per cent. Bull I is from no. 40's daughter, 44, the progenitor of strain B, and bull K is from no. 40's other daughter, 201, progenitor of strain A. The average of no. 40's daughters is 3.48 per cent. Bull F, her son, sired a daughter in each of the families testing 3.40 and 2.97 per cent respectively. The sire of bull F had daughters in both families testing 3.59 and 3.05 per cent,

respectively. He thus lowered the test of his daughters under his dam and her daughters as well as his sire's daughters.

By the same manner of comparison, it is seen that bull I from the strain B cow (test 3.37) produced daughters in the two families that tested 3.58 and 3.48 respectively. Judging from one daughter of his sire (3.89 per cent), bull I might have received a factor of high test from his sire. However, two other daughters of his sire in the low family test only 3.28, which evidence does not mark him as prepotent for high test as these two daughters'

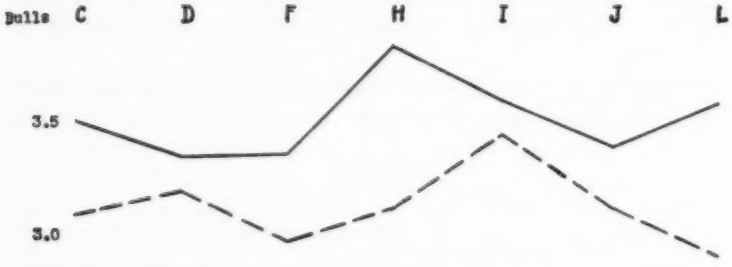

FIG. 6. SHOWING THE PER CENT OF FAT IN BOTH FAMILIES BY DAUGHTERS OF THE SEVERAL BULLS

Solid line represents the average per cent of fat in Altoana Pietertje A family of the daughters of the several bulls. Dotted line represents the average per cent of fat in the Fay M Family of the daughters of the several bulls. Each space represents 0.05.

tests were 3.24 and 3.32 per cent. Bull I did possess, evidently, by virtue of the dam's line the propensity for transmitting high tests. He is sire of the two highest testing cows in the low test family, viz.: No. 215 (3.76 per cent) and no. 212 (3.47 per cent). His third daughter, no. 211, in the low testing family tested 3.22 per cent. The first two are the only cases where the low test family crossed the average test line of the high test family.

Bull K having only one daughter does not throw any light on the subject. Although his dam, no. 201, produced one daughter having a test of 3.89 per cent, his (K) own daughter tested 3.45 per cent.

Figure 6 presents the average test of the seven bulls that have produced daughters in both families. They are given in the order of their use in the herd, the comparisons being made with respect to the relation to the original cows. In the case of C and D they were used in the herd during the same period of time. Since the daughters of these bulls may be and usually are products of more than one generation from the females, a progressive curve cannot be established. Compared to the original cow C raised the high and lowered the low test families a trifle; D lowered the high test and maintained the low test line; F lowered both families; H raised both; I raised both families; J lowered both lines; and L raised the high test slightly and lowered the low test considerably. Thus the bulls have apparently registered their own influence.

Thus with the limited data it is apparent that the bulls have influenced the test of the daughters but they have failed utterly to bring the two families together.

These results are somewhat contrary to the general belief and to genetically established facts. It is generally considered among dairy cattle breeders that the male is prepotent over the females to which he is bred. This is often the case in actual practise so far as milk yield is concerned, because the sire is usually the better bred animal. But the trite expression that "the bull is half of the herd" arises from the fact that the male has a numerical, rather than a biological advantage.

PRACTICAL CONSIDERATIONS

It has not been necessary to sacrifice on milk yield in order to maintain a comparatively high butter fat test, as is often assumed. The high testing family produced an average of 11,402.6 pounds of milk per lactation (not exceeding 365 days of which 14 of the 48 are by Advanced Registry) with an average test of 3.54 per cent of fat, while the low testing family produced 11,274.85 pounds of milk which tested 3.12 per cent, of which 16 of the 53 lactations are by Advanced Registry test. The difference in the milk yield of these two groups was negligible (275.8 pounds), but the average yearly fat production differed by 53 pounds.

The summary of the production records shows clearly the difference in the food value of the milk produced by the two families. The high testing family produced milk which averaged 12.47 per cent total solids, and the low testing family averaged 11.29 per cent. This would indicate that the milk produced by the high testing family contained 9.46 per cent more food value per unit.

Too great emphasis cannot be put upon the importance of foundation animals. Of course, it is not possible to know for a certainty how good an individual will prove to be as foundation stock, but every means of selection known, and especially the buying of cows out of families that have proved their ability to produce and transmit desirable qualities to their progeny should be used.

SUMMARY

In 1903 two Holstein cows were purchased. One of them had a butter fat test of 3.46 per cent and the other tested 3.25 per cent. These cows each produced two daughters, each of which formed a strain in the herd.

Altogether, Altoana Pietertje A (3.46 per cent) has produced 15 progeny that have milked through one or more 200 day lactation periods in the herd; and Fay M (3.25 per cent) has produced 20 progeny with similar performance. Represented here are six generations of Altoana Pietertje A and five generations of Fay M.

Even though sires have been used in common upon these two families, the tests remain as far apart today as originally. Only one in the high testing family has tested less than 3.25 per cent and only two in the low testing family (daughters of a son of a high test family cow) have tested above 3.46 per cent. The high test family has reached as high as 3.89 per cent and the low test family as low as 2.67 per cent in individuals.

Averaged by generations, the high test family maintains a test equal to the average for the breed, while the low test family usually is below the legal standard for fat. Low fat test in this family has behaved in dominant fashion. The one case of a

high test in the low test family (3.76) being from a daughter of a son from the high test family seems to be a case of Mendelian inheritance.

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EFFECT OF INGREDIENTS IN THE ICE CREAM MIX ON ITS FREEZING POINT*

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The freezing of the ice cream mix is one of the important steps in the manufacture of ice cream and while some work has been done on this subject, there has been no detailed study in which all influencing factors have been involved and the actual freezing points determined. In commercial freezing of the ice cream mix there is found a wide variation of brine temperatures and therefore a detailed study regarding the effect of ingredients on the freezing point of the ice cream mix will be of timely interest and value to the ice cream industry.

Baer (1) says that the time of freezing is affected only slightly by the amount of sucrose (cane sugar) in the mix. He explains that sucrose is in true solution in the mix and that the amount of sugar in the mix is directly proportional to length of time required to freeze. He found that the ice cream mix usually froze at 31°F.

Mojonnier and Troy (2) state that the greatest part of the depression in the freezing point of the ice cream mix is caused by the milk and cane sugars.

Bowen and Thomas (3) found that the freezing point of milk depended upon its composition.

The object of this experiment was to determine the effect the following ingredients have upon the freezing point of the ice cream mix: milk fat, sucrose, lactose, casein and albumen, gelatin, the production of acid, ageing and neutralizers.

The mixes were made up and frozen in a Hortvet Cryoscope which was equipped with a Centigrade thermometer graduated in degrees from 30 above, to 30 below zero, so that with the aid of

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[1] Student assistant.

a magnifying glass 1/10 of a degree could be read accurately. This thermometer had been checked with distilled water and definite percentage solutions of sucrose and was found accurate with 0.1°C. The freezing point was taken as the point at which the thermometer remained constant after there had been a rapid rise, due to the absorption of latent heat. The directions for use of the Hortvet Cryoscope as given by Emier and Amend (4) were followed. Duplicate determinations were made in all trials, and where there was variation in duplicate a triplicate determination made.

METHOD OF PROCEDURE

A standard mix was made up using the composition shown in table 1.

The skimmilk powder was dissolved in the cream and skimmilk and heated to 145°F. on a water bath, in which the surrounding water did not exceed 155°F. The gelatin and sugar mixture was then added and the entire mix held for twenty minutes at 145°F. and then cooled immediately.

A sample of this mix was tested on the Mojonnier tester for milk fat and total solids. The method of procedure on these tests was the same as outlined in the "Technical Control of Dairy Products" by Mojonnier and Troy (2). The sample was also tested for acidity, using Mann's Acid Test, as described in "Modern Methods of Testing Milk and Milk Products" by L. L. Van Slyke (5).

This standard mix was altered by change of various ingredients, but in all cases was balanced so as to contain the same proportion of ingredients except that ingredient which was under observation.

Table 2 shows that when the fat content is varied from 8 to 17 per cent, the freezing point remains constant. It appears from these results, that milk fat has no effect upon the freezing point of the ice cream mix. However, it must be considered that by adding fat or cream to a mix the sucrose content is reduced, but the lactose content will increase.

In this experiment the milk fat was increased by adding pure

milk fat and the sucrose content made up so that the percentage concentration of sucrose remained the same. There is a slight reduction of the lactose concentration in the mix but so slight as to come within the experimental error of the thermometer.

TABLE 1

INGREDIENT	AMOUNT OF INGREDIENT	PER CENT SERUM SOLIDS	PER CENT TOTAL SOLIDS	PER CENT MILK FAT
	grams			
Cane sugar	120		11.4	
Gelatin	5		0.47	
Skimmilk powder	60	5.7	5.7	
35 per cent cream	228	1.1	9.3	
Skimmilk	587	5.4	5.4	7.98
	1000	12.2	32.27	7.98

Lactose content = 6.2 per cent. Acidity = 0.28 per cent. Freezing point = −2.0°C.

TABLE 2

Effect of milk fat on freezing point of the standard ice cream mix

FREEZING POINT	PER CENT SUCROSE	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT MILK FAT
°C.				
−2.0	12.0	33.4	12.2	8.3
−2.0	12.0	39.7	10.9	17.6

TABLE 3

Effect of sucrose on freezing point of the standard ice cream mix

FREEZING POINT	PER CENT SUCROSE	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.0	12.0	33.4	12.2	8.3
−2.4	14.3	35.1	11.8	8.0
−2.7	15.8	36.3	11.6	7.9
−3.0	17.5	36.4	11.3	7.7
−3.6	19.2	39.2	11.1	7.5

The effect of sucrose on the freezing point is to decrease the freezing point. This decrease takes the form of a regular curve and the variation caused by 1 per cent is practically constant.

The other factors (lactose, milk fat, casein, lactic acid) in the mix were changed to such a slight degree, by the addition of sucrose, that they may be regarded as causing no change on the freezing point within 0.1°C.

TABLE 4

Effect of lactose on freezing point of the standard ice cream mix

FREEZING POINT	PER CENT LACTOSE	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.0	6.2	33.4	12.2	8.3
−2.2	9.2	35.4	14.8	8.0
−2.4	10.9	36.8	16.6	7.9
−2.7	12.7	37.1	18.3	7.7
−3.2	15.6	40.1	21.0	7.4

TABLE 5

Effect of age on freezing point of the standard ice cream mix

FREEZING POINT	TIME (AGE)	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.0	None	33.4	12.2	8.3
−2.0	3 days	33.4	12.2	8.3
−2.0	1 week	33.4	12.2	8.3
−2.0	2 weeks	33.4	12.2	8.3
−2.0	4 weeks	33.4	12.2	8.3

TABLE 6

Effect of egg albumen on freezing point of the standard ice cream mix

FREEZING POINT	PER CENT CASEIN	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.1	5.6	33.0	12.0	8.1
−2.3	8.4	35.0	14.6	7.8
−2.5	10.3	36.3	17.4	7.7
−2.6	12.2	37.7	18.3	7.5
−2.9	15.0	39.0	20.8	7.3

The results obtained from the effect of lactose on the freezing point of a standard ice cream mix shows that an increase in the lactose content lowers the freezing point of the standard ice

cream mix, provided the sucrose content is maintained at the original percentage.

It also shows that as the percentage of lactose is increased the variation caused by 1 per cent of lactose decreases and the curve obtained from the variation in the freezing point caused by lactose practically coincides with that made by sucrose. In the work on lactose, it was also found that when there was a large percentage of lactose in the mix the lactose would tend to crystalize out of solution before the freezing point was reached. This difficulty was overcome by having the ether at a fairly low temperature, thus inducing rapid freezing.

The results obtained from the effect of age on the freezing point of the ice cream mix shows that there was no variation in the freezing point. The acidity of the mix at start with 0.28 per cent and in 4 weeks 0.30 per cent. The mix was kept at a temperature between 33 and 34°F.

Table 6 shows that an increase in the egg albumen content (used as a substitute for casein) will produce a lowering of the freezing point. However, the effect is not so apparent as is the effect of sucrose and lactose.

In the work on sucrose, when the altered mix had a sucrose content of 19 per cent and a lactose content of 5.5 per cent, considering the water content only, the combined percentage of these two ingredients equals 39 per cent of the water content. This combined percentage lowered the freezing point of altered mix to −3.6°C. The sucrose and lactose content of the altered mix containing egg albumen was 17.5 per cent. This on a water content basis is 29.4 per cent of the water content.

Therefore, calculating on a water content basis it is safe to conclude that if a 39 per cent solution of sucrose and lactose lowered the freezing point to −3.6°C., then 29.4 per cent solution should lower it to −2.8°C. In the actual results the altered mix containing egg albumen froze at −2.9°C. The explanation of the lowering of the freezing point by a high concentration egg albumen is that the water content in the mix is reduced, making the sugar content more concentrated. By doing this, a lowering of the freezing point is brought about.

Plummer (6) states that colloidal solutions have a low osmotic pressure, and that the freezing point of the continuous phase is lowered very slightly and the boiling point is raised very slightly.

Gelatin which has had its jelling strength destroyed when added to a mix up to 4.1 per cent will not cause a variation in the freezing point. Gelatin when it makes up more than 2.5 per cent of a mix, will cause the mix to become highly viscous. This

TABLE 7

Effect of gelatin on the freezing point of the standard ice cream mix

FREEZING POINT	PER CENT GELATIN	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.1	0.5	33.0	12.0	8.1
−2.1	1.1	32.7	11.6	7.8
−2.1	1.7	32.4	11.4	7.7
−2.1	2.9	32.0	10.8	7.3
−2.1	4.1	31.6	10.2	6.9

TABLE 8

Effect of souring on the freezing point of the standard ice cream mix

FREEZING POINT	PER CENT AS LACTIC ACID	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−1.8	2.3	32.3	12.0	8.0
−2.0	2.8	32.3	12.0	8.0
−2.1	3.4	32.3	12.0	8.0
−2.3	8.2	32.3	12.0	8.0
−2.4	8.5	32.3	12.0	8.0

high viscosity will cause the temperature of the mix to be lowered so slowly that the point of latent heat absorption is not detectable. The only method by which the freezing point can be determined is to watch for the formation of small ice crystals which indicates that the mix is frozen. This shows very nicely that colloids have but slight effect on the freezing point which is well within the error of this work.

Results obtained on the effect of souring on the freezing point of the ice cream mix shows that souring lowers the freezing point of the mix, but that the lowering is not regular. It can be seen

that the variation due to 1 per cent increase in lactic acid is very regular, decreasing as the per cent of acid increases. The work on the effect of lactic acid was not extensive enough to point out any special reason for the irregularity in the lowering of the freezing point.

Results obtained in this work show that a small quantity of C.P. sodium bi-carbonate will lower the freezing point; 1.3 grams of sodium bicarbonate added to 100 grams of mix lowered the freezing point 0.5°C.

TABLE 9

Effect of neutralizer on the freezing point of the ice cream mix

FREEZING POINT	PER CENT LACTIC ACID	PER CENT TOTAL SOLIDS	PER CENT SERUM SOLIDS	PER CENT FAT
°C.				
−2.4	3.4	34.3	12.4	8.7
−2.9	2.0	34.3	12.4	8.7

SUMMARY

1. Milk fat has no effect on the freezing point of the ice cream mix.

2. Increasing the sucrose content of the ice cream mix will lower the freezing point in direct proportion to the amount of sucrose added to the mix.

3. As a percentage of sucrose is increased the variation caused by 1 per cent is constant.

4. The effect of increasing the lactose content of the ice cream mix is that of lowering the freezing point with the same regularity that the freezing point is lowered by sucrose.

5. The variation caused by 1 per cent of lactose steadily decreases as the total percentage of lactose in the mix increases.

6. Egg albumen (casein) does not affect the freezing point of the ice cream mix.

7. Gelatin that has had its jelling strength destroyed by heating does not affect the freezing point of the ice cream mix.

8. Souring lowered the freezing point of the ice cream mix but the lowering is not regular.

9. Neutralizer in the form of sodium bi-carbonate when added to the ice cream mix caused a lowering of the freezing point.

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SOY BEAN OILMEAL AND GROUND SOY BEANS AS PROTEIN SUPPLEMENTS IN DAIRY RATIONS*

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It has been a problem to develop on Indiana farms crops which would supply sufficient protein in the dairy ration. Up to this time cottonseed meal, linseed oilmeal and gluten meal have been the chief high protein concentrates that have been used in the dairy ration. As all of these feeds must be purchased and brought to the farm, a much smaller quantity of them has been fed than that which should be fed.

Recently there has been considerable interest in the growing of soy beans on Indiana farms, and inquiries have been very frequently made by Indiana dairymen as to whether soy beans could be used as protein supplements in the dairy ration.

A number of early feeding trials have been made comparing soy bean oilmeal and soy bean cake with cottonseed meal as a protein supplement in the dairy ration, and they were found equal to or superior to cottonseed meal for production. Similar results have been obtained with ground soy beans (1) (2) (3). At the Indiana station (4), it has been found that ground soy beans together with a mineral mixture might be supplemented with good results for purchased protein concentrates in the ration of hogs. Feeding trials conducted recently (5) indicate that ground soy beans can be supplemented for linseed oilmeal in the dairy ration, and that soy bean oilmeal has practically the same feeding value as linseed oilmeal for milk production.

It has been suggested by some feed manufacturers interested in soy bean products, that farmers might send their soy beans to the mills where the oil would be extracted and the oilmeal returned to the farmers, the miller accepting the oil for his part of the transaction.

In the feeding of ground soy beans, the preparation of this feed

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can be done entirely on the farm, as these beans can be ground or crushed for the grain mixture. Some difficulty has been reported in regard to grinding soy beans because of their oil content, but the addition of some oats to the soy beans during the grinding process practically eliminates this difficulty.

An experiment, divided into two parts, has recently been completed at the Purdue Experiment Station. The first part of this experiment was conducted to compare the value of soy bean oilmeal with linseed oilmeal as protein supplements in the grain ration of the dairy cow. The second part compared the value of ground soy beans with linseed oilmeal for milk and fat production.

TABLE 1

Chemical analyses of protein supplements used

TRIAL	CONCENTRATE	CHEMICAL ANALYSES					
		Moisture	Ash	Crude protein	Crude fiber	Nitrogen free extract	Fat
	Linseed oilmeal (Old process)	9.1	5.4	33.9	8.4	35.7	7.5
I	Soy bean oilmeal (Old process)	7.2	5.4	44.5	6.9	29.3	6.7
II	Soy bean oilmeal (New process)	6.6	7.1	44.5	7.2	32.4	2.2
III	Soy bean oilmeal (Old process)	9.1	5.7	35.8	6.6	37.0	5.7
IV	Ground soy beans	7.5	5.4	32.2	11.0	32.5	11.4
V	Ground soy beans	Same as trial IV					

The chemical analyses of the various protein supplements used in the experiment show the nutrient content given in table 1.

CONDUCT OF THE EXPERIMENT

Each trial of this experiment consisted of three twenty-eight-day periods which included a seven-day preliminary period, thus making an actual experimental feeding period of twenty-one days. The reversal method of feeding was used throughout these trials.

Production records

The milk produced by each cow was weighed and recorded after each milking, and composite samples, containing proportionate amounts of milk from each milking of each cow, were made and tested for butterfat content, every seven days.

Feeds

Grain. The grain rations fed throughout this experiment were the ordinary herd mixtures and were not uniform throughout the entire five trials. The grain ration during each trial, however, remained the same, with the exception of the changes in the high protein supplements, so that a direct comparison could be made between these supplements.

TABLE 2

Results of trial I

PERIOD	CONCENTRATE	MILK PRODUCTION	FAT	PER CENT FAT
		pounds	*pounds*	
1	Soy bean oilmeal	2178.5	76.5	3.54
3	Soy bean oilmeal	2150.2	78.5	3.65
Average		2164.3	77.5	3.59
2	Linseed oilmeal	2169.1	75.5	3.5

Roughage. Silage was carefully weighed to the cows at the rate of 3 pounds per day per 100 pounds body weight. Alfalfa hay was fed to the cows at the rate of 1 pound per day per 100 pounds body weight.

The grain ration was fed at the rate of 1 pound for every 3 pounds of 4 to 6 per cent milk produced and 1 pound for every 4 pounds of 3 to 4 per cent milk produced.

Trial I

In this trial, a comparison was made between old process linseed oilmeal and old process soybean oilmeal. The feeds were fed to three lots of cows, with three cows in each lot, one Jersey

and two Ayrshires or two Holsteins in each lot, so that the milk production and fat test would be similar. Table 2 shows the average amounts of milk and fat produced during each of the three feeding periods. The average production of milk and fat of the first and third periods, during which time the cows were fed soy bean oilmeal, is compared with the production of the second period, when the cows were fed linseed oilmeal.

From table 2 it can be seen that the average milk production resulting from each protein supplement fed was almost identical. The fat test, however, seemed to favor the soy bean meal over the linseed oilmeal by approximately 0.1 per cent. This gave a

TABLE 3

Results of trial II

PERIOD	CONCENTRATE	MILK PRODUCTION	FAT	PER CENT FAT
		pounds	pounds	
1	Soy bean oilmeal	2490.6	77.71	3.120
3	Soy bean oilmeal	2222.6	66.29	2.982
Average		2356.6	72.00	3.051
2	Linseed oilmeal	2335.0	74.46	3.188

higher total fat production by approximately 2.5 per cent, when soy bean oilmeal was fed than when linseed oilmeal was fed.

Trial II

In this trial, comparison was made between new process soy bean oilmeal and old process linseed oilmeal in regard to milk and fat production. Two lots of cows, with four cows in each lot, were fed by the reversal method. The data obtained is shown in table 3.

These figures indicate that the soy bean oilmeal caused approximately 1 per cent greater milk production while the linseed oilmeal produced approximately 3 per cent more fat than the soy bean oilmeal.

Trial III

In trial III, old process soy bean oilmeal was compared with old process linseed oilmeal. The cows in these lots, three cows in each lot, were allowed to run on pasture and during the latter part of the trial were given free access to racks of alfalfa hay. Table 4 shows the amount of milk and fat produced and the average fat test according to the protein concentrate fed.

These figures show that soy bean oilmeal produced slightly more milk (1.25 per cent) than the linseed oil meal while the fat production was practically the same, regardless of the kind of

TABLE 4

Results of trial III

PERIOD	CONCENTRATE	MILK PRODUCTION	FAT	PER CENT FAT
		pounds	*pounds*	
1	Soy bean oilmeal	2214.3	70.8	3.19
3	Soy bean oilmeal	2270.2	69.01	3.04
Average		2242.2	69.9	3.115
2	Linseed oilmeal	2213.4	69.35	3.13

protein supplement fed. The average fat test of the lots receiving soy bean oilmeal was practically the same as that of the lot receiving linseed oilmeal.

Summary. A general summary of these three feeding trials comparing soy bean oilmeal with linseed oilmeal showed:

1. That old process soy bean oilmeal gave better results than new process soy bean oilmeal.

2. Both kinds of soy bean oilmeal gave better results than linseed oilmeal in milk production.

3. New process soy bean oilmeal gave decidedly lower production in fat than old process soy bean oil meal and somewhat lower production in fat than linseed oilmeal.

4. That a correlation apparently exists between the chemical analyses of the concentrates and the amount of fat produced, as new process soy bean oilmeal (which was a chemically extracted meal) showed a decidedly lower percentage of fat than either old

process soy bean oilmeal or old process linseed oilmeal. The average fat test of the cows receiving new process soy bean oil meal was decidedly lower—almost 0.2 per cent—than that of the cows receiving linseed oilmeal.

5. The average fat test of the cows receiving old process soy bean oilmeal was slightly higher than that of those receiving linseed oilmeal.

Trial IV

In this trial, a comparison was made between two lots of four cows in each lot, to determine the relative feeding value of ground

TABLE 5

Results of trial IV

PERIOD	CONCENTRATE	MILK PRODUCTION	FAT	PER CENT FAT
		pounds	*pounds*	
1	Ground soy beans	2440.7	87.71	3.59
3	Ground soy beans	2317.7	82.68	3.56
Average		2379.2	85.19	3.58
2	Linseed oilmeal	2318.8	83.18	3.58

soy beans and old process linseed oilmeal. The beans used in this trial were not whole beans but were small and broken beans cleaned from soy beans that were to be used for seed. The average percentage composition of the soy beans showed that they were decidedly higher in fat but lower in protein than either the soy bean oilmeal or linseed oilmeal.

The average production of these two lots when fed ground soy beans as compared with linseed oilmeal is as shown in table 5.

These figures show that ground soy beans produced approximately 2.5 per cent more milk and from 2 to 2.5 per cent more fat than the linseed oilmeal. The fat test was the same, regardless of the protein concentrate fed.

Trial V

This trial was conducted with two lots of three lots each. The method of comparison was the same as in the other trials. The

figures in table 6 show the average production of milk and fat and the average percentage of fat of the two lots of cows when fed ground soy beans as compared with linseed oilmeal.

These figures are also in favor of the ground soy beans, there being a difference of approximately 3 per cent, in milk production, and 2.5 to 3 per cent in fat production in favor of the ground soy beans.

Summary. The data obtained in trials IV and V, when ground soy beans were compared with linseed oilmeal show:

1. That there was an average increase of between 2 to 3 per cent in both milk and fat production in favor of ground soy beans when compared with linseed oilmeal.

TABLE 6

Results of trial V

PERIOD	CONCENTRATE	MILK PRODUCTION	FAT	PER CENT FAT
		pounds	*pounds*	
1	Ground soy beans	1825.1	55.48	3.01
3	Ground soy beans	1735.6	52.2	3.00
Average		1780.3	53.84	3.024
2	Linseed oilmeal	1723.9	52.30	3.053

2. While the fat content of the ground soy beans was decidedly higher than that of the linseed oilmeal, the average butterfat test of the milk produced by cows receiving each of these protein supplements was practically identical.

CONCLUSIONS

The results of this experiment to determine the feeding value of soy bean oilmeal and ground soy beans compared with linseed oil meal as a protein supplement in the dairy ration show:

1. That soy bean oilmeal has a feeding value equal to that of linseed oilmeal.

2. That ground soy beans have a feeding value for milk and fat production from 2 to 3 per cent higher than linseed oilmeal.

3. That ground soy beans, while much lower in protein than soy bean oilmeal, contain a much greater percentage of fat and from this source probably obtain much of their feeding value.

4. Ground soy beans can become one of the best sources for protein in the ration of Indiana dairy cows.

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A BACTERIOLOGICAL STUDY OF THE HOMOGENIZING PROCESS IN MAKING ICE CREAM[1]

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INTRODUCTION

In a previous paper (1) a bacteriological study was made of the influence of various manufacturing operations upon the bacterial content of ice cream. One of the operations studied was homogenizing. In this operation, which takes but a short time to complete, an increase in bacterial count was noted in the majority of the samples studied. The increase noted in the mix was presumed to be due to two causes: First, bacterial contamination of the mix from the homogenizer, and, second, a breaking up of the clumps of bacteria as the mix passed through the homogenizer. It was to determine whether there is an actual breaking up of the bacterial clumps as the ice cream mix passes through the homogenizer that the present work was undertaken.

PREVIOUS WORK

Hammer and Sanders (2) made a bacteriological study of the influence of homogenizing the ice cream mix upon the bacterial count both with and without pressure. When the mix was run through the homogenizer without pressure, they found an increase in the numbers of bacteria in all cases. However, all the samples analyzed were from the first material passing through. This material would serve to partially free the machine from bacterial contamination so that subsequent material passing through should not be contaminated from this source to

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nearly as great an extent. When the mix was passed through the homogenizer under pressure, an increase in the number of bacteria was shown in all cases except one. The increase in the number of bacteria when the mix was homogenized under pressure was not as great in most cases as without pressure. They state that, "At least two factors are operating to change the bacterial content when the pressure is thrown on; first, the machine has been in operation longer and the contamination from it is becoming less; second, the agitation in the machine tends to break up any clumps that may be present and thus apparently increase the count."

Peterson and Tracy (3) in a study to determine the relative importance of each step in the manufacture of the mix say that, "The increase after homogenizing and freezing is probably due, for the most part, to the breaking up of the bacterial clusters, which results in a higher count by the plate method."

Fay and Olson (10) in their study of the homogenizing process state that, "In general, however, the increases were larger than the decreases, and the averages of all the counts showed an increase of 26 per cent after homogenizing. Such an increase in bacterial count is, no doubt, more apparent than real, being due chiefly to the breaking up of clusters of organisms each individual of which may give rise to a separate colony on an agar plate." From their study of this process they conclude that, "Homogenization of the mix usually causes an increase in the bacterial count as determined by the agar-plate method. Such an increase is probably due to the breaking up of clumps of bacteria."

PRESENT WORK

The present work was designed to determine whether there was a breaking up of the bacterial clumps during the process of homogenizing. The materials going into the ice cream mix were placed in a starter can and heated to 145°F. for thirty minutes. The mix was thoroughly stirred by mechanical paddles during the entire pasteurizing process which usually took from forty-five minutes to an hour to complete. After the mix had been pas-

teurized it was then passed through the homogenizer at 2000 pounds pressure. Samples were taken as the mix passed into the homogenizer and as it came out on the other side. The samples were always collected after about half the mix had passed through the homogenizer or toward the end but never at the beginning since it was desirable to reduce the bacterial contamination from the homogenizer to a minimum.

Method

The samples were analyzed by two methods; the plate method and the direct microscopic method. The numbers of bacteria by the plate method were determined as follows: 1 gram of the mix was weighed into an Erlenmeyer flask and 99 cc. of sterile physiological saline solution added to it. Suitable dilutions were made from this and plated on milk-powder agar (4). The dilutions used were 1:100; 1:1000 and 1:10,000. The plates were incubated at 37°C. and counted at the end of forty-eight hours. All plates were made in duplicate and the counts represent an average of the two best plates unless otherwise noted. All counts are therefore per gram and not per cubic centimeter.

Breed's direct microscopic method (5), (6), (7), (8) with slight modifications was used to determine the numbers of individual and of groups of bacteria present. The method used was as follows: 0.01 gram of the mix was weighed on each end of a clean slide, and spread evenly over an area of 1 square cm. The slides were made in duplicate so that for each sample of mix four 1 square cm. areas were made. After the slides had been dried they were immersed in xylol for five minutes to remove the fat. The surplus xylol was removed and the slides dried again. They were then fixed in 95 per cent alcohol for twenty minutes and dried. After this they were stained for thirty seconds in Loeffler's alkaline methylene blue and decolorized to a light blue in 95 per cent alcohol. The bacteria stained a deeper blue than the rest of the material. Because of the larger amount of fat present in ice cream the fields are more pitted than in the case of milk. However, satisfactory preparations for comparison and other

detailed information may be obtained. Twenty-five or more fields from each area were counted and averaged. This made a total of 100 fields for each sample. The counting was done under a 1.9 mm. oil-immersion lens. The factor used was 500,000 and was derived as follows: Diameter of the field was 0.16 mm. By substituting in the following equation

$$\frac{x}{\pi R^2} \times 100 = y$$

where x equals the area of the smear in square millimeters, R the radius of the field in millimeters whence πR^2 equals the area, and y equals the factor necessary to transform the number of bacteria found in one field of the microscope into terms of bacteria per gram. Substituting we get

$$\frac{1 \times 100}{3.1416 \times (0.08)^2} \times \frac{100}{1} = 500{,}000$$

Since a hundred or more fields from each sample were counted, it was necessary to find the average per field and multiply this average by the factor. This was done as follows:

$$\frac{500{,}000}{n} \times m = \text{number of bacteria per gram}$$

when n = the number of fields counted and m the total number of bacteria found in n fields.

An explanation is necessary of the terms "individuals" and "groups." By the term "individuals" is meant the total number of bacteria including isolated single bacteria and the individual bacteria in groups (any organism in the process of division was counted as two individuals); thus the count under the column labeled individuals represents a total of all the bacterial cells in an average of 100 fields times the factor. The column labeled "groups" represents a somewhat different meaning than is usually ascribed to this term and should be thoroughly understood by anyone wishing to interpret the data correctly. By this term is meant those bacteria, either individuals or groups, so located in the microscopic field that in the opinion of the observer would, if alive and plated on suitable medium, form one bacterial colony.

Thus a single individual if sufficiently isolated would count as a "group" or several individuals if sufficiently close to each other would constitute a "group." In the process of counting many

TABLE 1

Comparison of plate count and direct microscopic count before and after homogenizing

DATE	PLATE COUNT		DIRECT MICROSCOPIC COUNT			
			Before		After	
	Before	After	Individuals	"Groups"	Individuals	"Groups"
1922						
10/3	5,800	10,000	1,980,000	1,160,000	3,330,000	3,110,000
10/10	17,000	18,000	1,950,000	945,000	2,200,000	2,000,000
10/17	10,000	25,000	1,450,000	1,100,000	7,335,000	7,120,000
10/23	46,500	71,000	2,400,000	1,890,000	3,000,000	2,900,000
10/30	12,500	31,000	2,865,000	2,200,000	3,825,000	3,450,000
11/3	7,600	16,000	3,400,000	2,500,000	4,100,000	3,800,000
11/10	18,000	30,000	2,780,000	1,650,000	4,350,000	4,000,000
11/15	6,500	23,000	2,800,000	1,650,000	2,700,000	2,320,000
12/20	19,000	88,000	2,500,000	1,560,000	4,315,000	4,000,000
1923						
3/16	8,100	4,600	2,400,000	1,550,000	3,725,000	3,230,000
3/21	23,000	67,000	3,340,000	1,880,000	3,885,000	3,600,000
4/10	4,500	8,000	2,330,000	1,700,000	3,000,000	2,565,000
4/18	5,500	15,000	2,530,000	1,585,000	2,680,000	2,195,000
4/19	12,000	18,000	3,000,000	2,600,000	2,720,000	2,340,000
10/8	16,000	38,000	1,700,000	1,560,000	1,850,000	1,700,000
10/12	3,000	3,800	1,600,000	1,300,000	1,900,000	1,700,000
10/19	24,000	83,000	490,000	455,000	465,000	435,000
10/25	14,000	60,000	850,000	750,000	1,600,000	1,500,000
11/9	69,000	75,000	545,000	450,000	720,000	600,000
11/13	6,500	19,000	2,000,000	1,500,000	2,200,000	2,000,000
11/15	3,600	6,500	665,000	570,000	570,000	555,000
11/22	12,500	170,000	3,965,000	2,000,000	2,420,000	2,160,000
11/28	11,000	45,000	2,100,000	1,600,000	3,500,000	3,200,000
12/3	2,500	300,000	1,850,000	1,450,000	3,500,000	3,200,000
1924						
2/20	4,400	6,000	2,665,000	1,440,000	2,700,000	1,750,000

objects were encountered about which there might be a reasonable doubt as to whether they were bacteria or not. In all such cases, they were not counted as bacteria.

Results

The results of the experiment are set forth in the tables that follow. In table 1 a comparison is made of the data secured from the plate count and the direct microscopic count. In the second column under plate count are listed the number of bacteria found in the ice cream mix before it was homogenized, but after it had been pasteurized at 145°F. for thirty minutes. In the third column are listed the numbers of bacteria found on the plates after the ice cream mix had passed through the homogenizer under 2000 pounds pressure.

In column 4 are listed the counts of individual bacteria of the same mix under the same conditions as in column 2 above except that the number of bacteria have been determined by the modified direct microscopic method. In column 5 are listed the "groups" of bacteria of this same mix as determined by the modified microscopic method.

In columns 6 and 7 are listed respectively the counts of the same mix as listed in column 3 above except that the "individuals" and "groups" have been determined by the direct microscopic method slightly modified. In other words the same mix under identical conditions has been analyzed by two different bacteriological methods both before and after the mechanical operation of homogenizing to determine the effect this process had on the bacterial content of the mix. A comparison of the plate count and direct microscopic count before and after homogenizing are set forth in table 1.

Discussion

A comparison of columns 2 and 3 of table 1 shows that there is an increase in the number of bacteria after homogenizing as compared with before homogenizing as determined by the plate count in all cases except one. In some cases the increase is large while in other cases it is small.

A comparison of columns 4 and 6 tabulating individuals before and after homogenizing, as determined by the direct microscopic method, shows, that in 20 out of 25 or 80 per cent of the samples

there is an increase; while in 5 out of 25 or 20 per cent of the samples, there is a decrease in the number of individuals after as compared with before homogenizing. The same relationship as just stated for individuals also holds true for "groups" tabulated in columns 5 and 7. It will thus be seen that, in the majority of cases, there is an increase in the bacterial count, as determined by both the plate and direct microscopic method, after the mix has passed through the homogenizer.

RELATIONSHIP BETWEEN INDIVIDUALS, "GROUPS" AND GROUP SIZE

The individuals, "groups," and group size are recorded in table 2. What is meant by individuals and "groups" has been defined earlier in this paper. However, it was thought advisable to make further tabulations concerning the number of groups and group size, since we are concerned primarily in trying to determine the cause of the increase of the number of bacteria as determined by the plate count and as reported by previous investigators.

For this reason a record was made of the number of groups and the size of each group as the count was made. In column 7 are tabulated all the groups of two and in column 8 all the groups of three or more. Total groups of two or more are listed in column 6 while the average size of groups of two or more are listed in column 9. The complete record of this detailed information is given in table 2.

Discussion

The size of groups most common above two are three, four and five in the order mentioned. Groups of eight are not infrequent while the size of a few groups ran as large as sixty or more. However, the larger groups occurred rather infrequently in the ice cream mix.

It will be noticed that there is a considerable difference between the average size of "groups" in column 5 and the average size of groups of two or more in column 9, the average size of groups in column 9 being considerably larger than in column 2. This is

TABLE 2

Tabulation of individuals, "groups" and group size

DATE		NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE OF GROUPS OF TWO OR MORE
						Groups of two	Groups of three or more	
1922								
10/ 3	B*	1,980,000	1,160,000	1.70	39	19	20	3.8
	A	3,330,000	3,110,000	1.07	28	17	11	3.0
10/10	B	1,950,000	945,000	2.06	55	26	29	4.7
	A	2,200,000	2,000,000	1.10	39	34	5	2.3
10/17	B	1,450,000	1,100,000	1.31	30	11	19	3.0
	A	7,335,000	7,120,000	1.03	14	4	10	2.9
10/23	B	2,400,000	1,890,000	1.27	44	16	28	5.0
	A	3,000,000	2,900,000	1.04	5	3	2	2.4
10/30	B	2,865,000	2,200,000	1.32	78	31	47	3.0
	A	3,825,000	3,450,000	1.17	40	27	13	2.5
11/ 3	B	3,400,000	2,500,000	1.36	65	19	46	3.8
	A	4,100,000	3,800,000	1.06	41	21	20	2.4
11/10	B	2,780,000	1,650,000	1.70	106	34	72	3.5
	A	4,350,000	4,000,000	1.08	35	16	19	2.8
11/15	B	2,800,000	1,650,000	1.70	104	33	71	4.0
	A	2,700,000	2,320,000	1.16	23	10	13	3.0
12/20	B	2,520,000	1,560,000	1.42	92	45	47	3.1
	A	4,315,000	4,000,000	1.07	38	20	18	2.6
1923								
3/16	B	2,400,000	1,550,000	1.54	95	32	62	3.1
	A	3,725,000	3,230,000	1.15	58	30	28	2.3
3/21	B	3,340,000	1,880,000	1.77	78	40	38	3.3
	A	3,885,000	3,600,000	1.08	42	29	13	2.4
4/10	B	2,330,000	1,700,000	1.43	76	44	32	3.0
	A	3,000,000	2,565,000	1.17	60	39	21	2.4
4/18	B	2,530,000	1,585,000	1.69	89	40	49	3.2
	A	2,680,000	2,195,000	1.22	36	27	9	3.1
4/19	B	3,000,000	2,600,000	1.15	46	27	19	3.0
	A	2,720,000	2,340,000	1.11	51	37	14	2.5

TABLE 2—*Continued*

DATE		NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE OF GROUPS OF TWO OR MORE
						Groups of two	Groups of three or more	
1923								
10/ 8	B	1,700,000	1,560,000	1.09	30	27	3	2.2
	A	1,850,000	1,700,000	1.08	23	20	3	2.1
10/12	B	1,600,000	1,300,000	1.23	44	35	9	2.2
	A	1,900,000	1,700,000	1.11	29	24	5	2.1
10/19	B	490,000	455,000	1.07	17	16	1	2.2
	A	465,000	435,000	1.06	9	8	1	2.1
10/25	B	850,000	750,000	1.13	27	21	6	2.3
	A	1,600,000	1,500,000	1.06	21	21	0	2.0
11/ 9	B	545,000	450,000	1.21	18	17	1	2.1
	A	720,000	600,000	1.20	25	23	2	2.0
11/13	B	2,000,000	1,500,000	1.33	67	61	6	2.4
	A	2,200,000	2,000,000	1.10	37	36	1	2.0
11/15	B	665,000	570,000	1.17	15	13	2	2.5
	A	570,000	555,000	1.02	20	15	5	2.2
11/22	B	3,965,000	2,000,000	1.98	173	127	46	4.3
	A	2,420,000	2,160,000	1.12	33	33	0	2.0
11/28	B	2,100,000	1,600,000	1.31	99	87	12	2.1
	A	3,200,000	2,950,000	1.06	63	61	2	2.0
12/ 3	B	1,850,000	1,450,000	1.27	72	66	6	2.1
	A	3,500,000	3,200,000	1.09	44	43	1	2.0
1924								
2/20	B	2,665,000	1,440,000	1.85	112	76	36	3.1
	A	2,700,000	1,750,000	1.54	105	76	29	2.4

* B = before mix passed through homogenizer; A = after mix passed through homogenizer.

readily understood when the method of computing these averages is explained.

The method of computing the average size of the "group" in

column 5 was by dividing the total number of individuals by the total number of "groups;" while the method used in computing the average size of the groups of two or more in column 9 was by finding the total number of individuals occurring in the groups alone (not including separate individuals) and dividing this sum by the total number of actual groups. Both methods of computation show that there is a decrease in the size of groups after the mix has passed through the homogenizer.

Since all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of the clumps of bacteria in the mix.

A STUDY OF THE INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK

When the work had been completed showing the influence of homogenizing on ice cream, it was thought that a similar study of a few samples of milk might prove interesting and throw some light on the problem. Accordingly 5 samples of milk were studied with this in view. At first it was thought advisable to secure samples of fresh unpasteurized milk having large numbers of bacteria present.

Samples of milk coming into the dairy were analyzed until one was found with a high bacterial plate count. This was run but it was not found to be satisfactory for this work as will be seen later. A composite sample of milk as delivered at the dairy was found most satisfactory and was used in the other 4 cases.

Procedure

A 10-gallon can of fresh unpasteurized milk containing milk from many different farms was taken from the vat at the College dairy. This can of milk was then dumped into the starter can leading to the homogenizer and a sample taken. It was then passed through the homogenizer without pressure. The homogenizer pressure was then raised to 1000, 1500, 2000 and 2500 pounds pressure per square inch respectively and samples taken as it was leaving the homogenizer at these various pressures.

These samples were then taken immediately to the laboratory and analyzed according to the direct and plate methods as previously described. Previous to homogenization, the homogenizer was rinsed with sterile water and a sample of this rinse water was collected and analyzed by the plate method.

TABLE 3

Sample 1, February 28, 1924

OPERATION	PLATE COUNT	NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	REMARKS
A	3,500,000	50,000,000*	2,520,000	19.84	25 fields
B	19,000,000	166,300,000*	Bacteria spread evenly over entire field		
C	14,000,000		Bacteria spread evenly over entire field		
D	26,000,000		Bacteria spread evenly over entire field		
E	23,000,000		Bacteria spread evenly over entire field		
F	35,400,000†		Bacteria spread evenly over entire field		

Water from homogenizer, 1500 bacteria per cubic centimeter.

* Twenty-five fields counted.

† Only one plate counted.

Explanation of letters for all tables

Operation A = fresh unpasteurized milk from vat.
Operation B = same milk passed through homogenizer without pressure.
Operation C = same milk passed through homogenizer at 1000 pounds pressure.
Operation D = same milk passed through homogenizer at 1500 pounds pressure.
Operation E = same milk passed through homogenizer at 2000 pounds pressure.
Operation F = same milk passed through homogenizer at 2500 pounds pressure.

TABLE 4

Sample 2, March 13, 1924

OPERATION	PLATE COUNT	NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE OF GROUPS
						Groups of two	Groups of three or more	
A	840,000	2,700,000	1,240,000	2.17	142	85	57	2.8
B	1,000,000	4,500,000	1,580,000	2.84	154	64	90	5.0
C	2,115,000	2,570,000	1,800,000	1.43	101	72	29	2.5
D	2,300,000	2,400,000	1,330,000	1.80	97	69	28	2.5
E	2,600,000	1,830,000	1,380,000	1.32	78	64	14	2.2
F	2,800,000	2,500,000	1,450,000	1.73	77	54	23	2.4

Water from homogenizer, 6 bacteria per cubic centimeter.

TABLE 5

Sample 3, March 20, 1924

OPERATION	PLATE COUNT	NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE OF GROUPS
						Groups of two	Groups of three or more	
A	25,000	450,000*	320,000	1.40	5	4	1	3.6
B	68,000	550,000	300,000	1.83	19	15	4	2.5
C	124,000	360,000	290,000	1.24	10	9	1	2.3
D	78,000	340,000	295,000	1.15	8	7	1	2.1
E	77,000	350,000	315,000	1.11	7	7	0	2.0
F	86,000	350,000*	300,000	1.16	3	3	0	2.0

Water from homogenizer, 125,000 bacteria per cubic centimeter.
* Fifty fields counted.

TABLE 6

Sample 4, March 26, 1924

OPERATION	PLATE COUNT	NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE OF GROUPS
						Groups of two	Groups of three or more	
A	435,000	1,870,000	675,000	2.77	38	33	15	6.8
B	2,300,000	3,500,000	1,500,000	2.33	182	119	63	3.1
C	2,200,000	4,200,000	2,400,000	1.75	194	123	71	2.8
D	2,600,000	4,500,000	2,400,000	1.87	220	135	85	2.9
E	2,800,000	3,000,000	2,320,000	1.29	145	102	43	2.5
F	3,400,000	2,300,000	1,460,000	1.57	120	67	43	2.5

Water from homogenizer, 140,000 bacteria per cubic centimeter.

TABLE 7

Sample 5, March 27, 1924

OPERATION	PLATE COUNT	NUMBER OF INDIVIDUAL BACTERIA	NUMBER OF "GROUPS"	AVERAGE SIZE OF "GROUPS"	TOTAL GROUPS OF TWO OR MORE	NUMBER OF		AVERAGE SIZE of GROUPS
						Groups of two	Groups of three or more	
A	250,000	1,950,000	780,000	2.5	39	21	18	7.0
B	400,000	2,400,000	800,000	3.0	89	53	36	4.9
C	72,000	1,700,000	875,000	1.94	71	40	31	3.2
D	105,000	1,400,000	830,000	1.70	69	54	15	2.5
E	285,000	1,400,000	950,000	1.47	56	37	19	2.6
F	560,000	1,700,000	1,000,000	1.70	56	34	22	2.1

Water from homogenizer, 1800 bacteria per cubic centimeter.

Results

The results of this experiment are recorded in tables 3 to 7. In table 3 are recorded the results obtained by using the sample of milk containing a large number of bacteria. Only 25 fields were counted because the bacteria were so numerous. In this sample there were so many large clumps and the organisms so many that the number of bacteria in the large clumps had to be estimated. For this reason it was thought best to work with milk containing fewer bacteria. This would enable a more accurate counting of the bacteria in the groups.

In tables 4, 5, 6 and 7 are recorded the results from the composite samples of milk. The numbers of bacteria by the plate count and the direct microscopic count are recorded the same as for the ice cream mix. The same information as to individuals, "groups," average size of "groups," etc., is also contained in the tables.

The operations are lettered and have the following meaning: Operation "A," fresh unpasteurized milk after being placed in the starter can and just before passing through the homogenizer; operation "B," the same milk after passing through the homogenizer without pressure; operation "C," same milk after passing through the homogenizer at 1000 pounds pressure; operation "D," same milk after passing through the homogenizer at 1500 pounds pressure; operation "E," same milk after passing through the homogenizer at 2000 pounds pressure; operation "F," same milk after passing through the homogenizer at 2500 pounds pressure.

A detailed record of the results is set forth in tables 3 to 7.

INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK

Discussion

A careful study of the data shows that the clumps of bacteria in milk are broken up during the process of homogenizing. This is especially marked in the first sample of milk run. In this sample large clumps of bacteria together with many smaller

clumps were scattered throughout the entire smear before the milk was homogenized. After homogenizing, however, the bacteria were evenly distributed throughout the entire smear and the size of the clumps greatly reduced in size. The same held true for the other samples homogenized but to a less degree because there were fewer large groups and not nearly as many organisms present. In all cases where the milk passed through the homogenizer under pressure there is a decrease in the size of the groups.

The data also show that the homogenizer is a source of contamination for in all cases there is an increase in both the plate and microscopic counts after the milk has passed through the homogenizer. It is true that a part of this increase might be attributed to the breaking up of clumps and no doubt was to a slight extent but the large increase should then be maintained throughout which is not the case. Another interesting thing is that the average size of the "groups" increases in most cases after the milk has passed through the homogenizer, which would indicate contamination from the homogenizer. The average size of groups decreases slightly in most cases with an increase of pressure. The decrease is gradual which would indicate that as the groups get smaller they are harder to break up.

The above data show that homogenizing has the same influence on the bacterial groups in milk as it does on the bacteria groups in the ice cream mix.

A STUDY OF THE TYPES OF BACTERIA FOUND IN THE HOMOGENIZER AND IN THE ICE CREAM MIX BEFORE AND AFTER HOMOGENIZING

In making a bacteriological study of the homogenizing process milk-powder agar was used. By the use of this medium interesting data as to the types of bacteria present in the homogenizer were obtained. By using this medium it was thought that some data could also be obtained as to the types of bacteria present in the mix before and after homogenizing and also the types of bacteria present in the homogenizer.

Types of bacteria present

The medium was made according to directions and the bacterial colonies counted and recorded according to the system described by Ayers and Mudge (4) on page 579 of their article, viz.:

1. A total count was made.
2. The strong acid-forming colonies were then counted. The strong acid-forming colonies are defined as, "Those with a cloudy zone about them or a slight hazy edge."
3. Weak acid-forming colonies were next counted. All colonies showing acid but without a cloudy zone or a slight hazy edge about them were considered as weak acid-forming colonies.
4. The plate was then flooded with a 5 per cent solution of acetic acid and all colonies showing the characteristic clearing about the colony were counted as peptonizers.
5. The difference between the sum of the strong acid-forming, the weak acid-forming, and the peptonizing colonies and the total count were classified as alkali formers and inert colonies.

By the use of milk-powder agar and classifying the colonies according to the above scheme, one is able to obtain a pretty good bacteriological picture of the types of colonies present.

Results

The types of colonies found in the water from the homogenizer are set forth in table 8. The homogenizer was thoroughly rinsed with sterile hot water by passing several gallons through it and collecting the last part of the water and plating it on the milk-powder agar. The results show a predominance of alkaline and inert colonies with weak acid colonies next in number with nearly as many peptonizers. The strong acid-forming colonies are few in number as compared to the rest.

In table 9 are recorded the results of plating out a sample of the mix just after it had been pasteurized and before it passed through the homogenizer. The procedure followed in the College dairy is to put the materials for the mix in a starter can and pasteurize and mix it at one and the same time after which it is passed through the homogenizer. The data in table 9 are

collected from samples taken as the mix is ready to pass on to the process of homogenizing.

We find a predominance of alkali and inert colonies, 42.82 per cent; with weak acid-forming colonies coming next, 34.75 per

TABLE 8

Type of colonies of bacteria found in water from homogenizer

DATE	TOTAL COUNT	STRONG ACID		WEAK ACID		PEPTONIZERS		ALKALINE AND INERT BACTERIA	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1922									
10/3	8					6	75.00	2	25.00
10/10	2,800	700	25.00	600	21.43	600	21.43	900	32.14
10/17	2,200	150	6.82	1,000	45.45	500	22.72	550	25.00
10/23	5,000	500	10.00	2,000	40.00	1,500	30.00	1,000	20.00
10/30	3,900			360	9.23	350	8.97	3,190	81.80
11/3	8,000	500	6.25	5,000	62.50	1,900	23.75	600	7.50
11/10	450			100	22.22	200	44.44	150	33.33
11/15	15					10	66.66	5	33.33
12/20	7,500			4,500	60.00	2,000	26.66	1,000	13.33
1923									
3/16	No water saved								
3/21	No water saved								
4/10	8,500					500	5.88	8,000	94.12
4/18	300	18	6.00	6	2.00	80	26.66	196	65.33
4/19	2,000	10	0.50			10	0.50	1,980	99.00
10/8	8,500			4,000	47.05	1,000	11.76	3,500	41.17
10/12	5			4	80.00	1	20.00	0	
10/19	5,000	500	10.00	3,500	70.00	800	16.00	200	4.00
10/25	4,000			500	12.50	2,500	62.50	1,000	25.00
11/9	43,000	3,000	6.97	18,000	41.86	10,000	23.25	12,000	27.90
11/13	Spreader								
11/15	650			85	13.07	400	61.54	165	25.38
11/22	2			2	100.00				
11/28	18,000			3,500	19.44	5,000	27.77	9,500	52.77
12/3	140,000	10,000	7.14	70,000	50.00	45,000	32.14	15,000	10.71
1924									
2/20	315			85	26.98	130	41.27	100	31.74
Average.....	.....		3.58		32.90		29.50		34.02

cent; with peptonizers next, 17.41 per cent; and strong acid-forming colonies last with 5.07 per cent.

Table 10 shows the type of colonies prevailing in the mix after it has passed through the homogenizer. It will be noticed that

TABLE 9

Type of colonies of bacteria in mix before homogenizing

DATE	TOTAL COUNT	STRONG ACID		WEAK ACID		PEPTONIZER		ALKALINE AND INERT BACTERIA	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1922									
10/3	5,800	450	7.76	3,800	65.51	500	8.62	1,050	18.11
10/10	17,000	2,200	12.94	2,700	15.88	1,200	7.05	10,900	64.12
10/17	10,000*	3,000	30.00			2,000	20.00	5,000	50.00
10/23	46,500			25,000	53.76	6,500	13.98	15,000	32.25
10/30	12,500*			6,300	50.40	4,000	32.00	2,200	17.60
11/3	7,600*			4,400	57.90	500	6.58	2,700	35.52
11/10	18,000			13,000	72.22	2,900	16.11	2,100	11.66
11/15	6,500	50	0.77	2,500	38.46	800	12.30	3,150	48.46
12/20	19,000			6,000	31.58	4,500	23.68	8,500	44.73
1923									
3/16	8,100			100	1.23	450	5.55	7,550	93.21
3/21	23,000	2,300	10.00	650	2.82	4,000	17.39	16,050	69.79
4/10	4,500					700	15.55	3,800	84.45
4/18	5,500	1,400	25.45	450	8.18	1,500	27.27	2,150	39.09
4/19	12,000	450	3.75	300	2.50	1,400	11.66	9,850	82.08
10/8	16,000	800	5.00	11,000	68.75	1,800	11.25	2,400	15.00
10/12	3,000	800	26.66	200	6.66	800	26.66	1,200	40.00
10/19	24,000			11,000	45.83	4,000	16.66	9,000	37.50
10/25	14,000			2,500	17.85	5,000	35.71	6,500	46.44
11/9	69,000*			20,000	29.00	9,000	13.00	40,000	58.00
11/13	6,500			2,500	38.46	1,500	23.07	2,500	38.46
11/15	3,600			1,300	36.11	1,100	30.55	1,200	33.33
11/22	12,500			9,300	74.40	1,600	12.80	1,600	12.80
11/28	11,000	500	4.54	7,500	68.18	2,000	18.18	1,000	9.09
12/3	2,500			1,000	40.00	300	12.00	1,200	48.00
1924									
2/20	4,400			1,900	43.16	700	15.90	1,800	40.90
Average.....			5.07		34.75		17.41		42.82

* Only one plate counted.

the difference between the number of colonies in the alkali and inert group, 36.43 per cent, and weak acid-forming group, 35.69 per cent is not so great as in table 9. It will be further noted

TABLE 10

Type of colonies of bacteria in mix after homogenizing

DATE	TOTAL COUNT	STRONG ACID		WEAK ACID		PEPTONIZER		ALKALINE AND INERT BACTERIA	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1922									
10/3	10,000	1,900	19.00	4,900	49.00	800	8.00	2,400	24.00
10/10	18,000	3,900	21.66	9,100	50.55	550	3.05	4,450	24.72
10/17	25,000*	1,000	4.00	15,000	60.00	4,000	16.00	5,000	20.00
10/23	71,000	3,000	4.22	30,000	42.25	9,000	12.66	29,000	40.84
10/30	31,000	2,000	6.45	14,000	45.16	1,500	4.83	13,500	43.54
11/3	16,000*	600	3.75	10,000	62.50	1,600	10.00	3,800	23.75
11/10	30,000			25,000	83.33	1,500	5.00	3,500	11.66
11/15	23,000	5,000	21.74	8,000	34.77	3,000	13.04	7,000	30.43
12/20	88,000	500	0.57	45,000	51.13	22,000	25.00	20,500	23.30
1923									
3/16	4,600	150	3.26	700	15.21	1,600	34.78	2,150	46.74
3/21	67,000	1,300	1.94	300	0.44	1,500	2.24	63,900	95.37
4/10	8,000	550	7.00			450	5.62	7,000	87.50
4/18	15,000	3,000	20.00	1,800	12.00	4,500	30.00	5,700	38.00
4/19	18,000	2,500	13.88	900	5.00	1,100	6.12	13,500	75.00
10/8	38,000	1,400	3.68	21,000	55.26	1,900	5.00	13,700	36.05
10/12	3,800			1,800	47.37	1,500	39.47	500	13.15
10/19	83,000	5,000	6.02	28,000	33.73	22,000	26.50	28,000	33.73
10/25	60,000*			10,000	16.66	40,000	66.66	10,000	16.66
11/9	75,000*	2,000	2.66	16,000	21.33	16,000	21.33	41,000	54.66
11/13	19,000	500	2.63	4,500	23.68	7,500	39.47	6,500	34.21
11/15	6,500			2,000	30.77	3,000	46.15	1,500	23.07
11/22	170,000	2,000	1.18	40,000	23.53	45,000	26.47	83,000	48.82
11/28	45,000	4,000	8.88	29,000	64.44	500	1.11	11,500	25.55
12/3	300,000	5,000	1.66	85,000	28.33	170,000	56.66	40,000	13.33
1924									
2/20	6,000			2,000	33.33	2,400	40.00	1,600	26.66
Average.....			6.16		35.69		21.81		36.43

* Only one plate counted.

that the number of colonies of peptonizers has increased from 17.41 per cent in table 9 to 21.81 per cent in table 10 while the number of strong acid-forming colonies shows but a slight increase.

TABLE 11

A comparison of the types of colonies before and after homogenizing

(Plate count—milk-powder agar)

DATE	TOTAL COUNT		STRONG ACID		WEAK ACID		PEPTONIZERS		ALKALINE AND INERT		WATER FROM HOMOGENIZER
	Before	After	Before	After	Before	After	Before	After	Before	After	
1922											
10/3	5,800	10,000	450	1,900	3,800	4,900	500	800	1,050	2,400	8
10/10	17,000	18,000	2,200	3,900	2,700	9,100	1,200	550	10,900	4,450	2,800
10/17	10,000*	25,000*	3,000	1,000		15,000	2,000	4,000	5,000	5,000	2,200
10/23	46,500*	71,000		3,000	25,000	30,000	6,500	9,000	15,000	29,000	5,000
10/30	12,500*	31,000		2,000	6,300	14,000	4,000	1,500	2,200	13,500	3,900
11/3	7,600	16,000*		600	4,400	10,000	500	1,600	1,700	3,800	8,000
11/10	18,000	30,000			13,000	25,000	2,900	1,500	2,100	3,500	450
11/15	6,500	23,000	50	5,000	2,500	8,000	800	3,000	3,150	7,000	15
12/20	19,000	88,000		500	6,000	45,000	4,500	22,000	8,500	20,500	7,500
1923											
3/16	8,100	4,600		150	100	700	450	1,600	7,550	2,150	
3/21	23,000	67,000	2,300	1,300	650	300	4,000	1,500	16,150	63,900	
4/10	4,500	8,000		550			700	450	3,800	7,000	8,500
4/18	5,500	15,000	1,400	3,000	450	1,800	1,500	4,500	1,150	5,700	300
4/19	12,000	18,000	450	2,500	300	900	1,400	1,100	9,850	13,500	2,000
10/8	16,000	38,000	800	1,400	11,000	21,000	1,800	1,900	2,400	13,700	8,500
10/12	3,000	3,800	800		200	1,800	800	1,500	1,200	500	30
10/19	24,000	83,000		5,000	11,000	28,000	4,000	22,000	9,000	28,000	5,000
10/25	14,000	60,000*			2,500	10,000	5,000	40,000	6,500	10,000	4,000
11/9	69,000*	75,000*		2,000	20,000	16,000	9,000	16,000	40,000	41,000	43,000
11/13	6,500	19,000		500	2,500	4,500	1,500	7,500	2,500	6,500	
11/15	3,600	6,500			1,300	2,000	1,100	3,000	1,200	1,500	650
11/22	12,500	170,000		2,000	9,300	40,000	1,600	45,000	1,600	83,000	2
11/28	11,000	45,000	500	4,000	7,500	29,000	2,000	500	1,000	11,500	18,000
12/3	2,500	300,000		5,000	1,000	85,000	300	170,000	1,200	40,000	140,000
1924											
2/20	4,400	6,000			1,900	2,000	700	2,400	1,800	1,600	315

* Only one plate counted.

STATISTICAL ANALYSIS OF THE SIGNIFICANCE OF CHANGES DUE TO HOMOGENIZING[2]

In table 11 is set down the total number of bacteria in the mix before and after homogenizing, also the number of each of the different types before and after the homogenizing process. This comparative data permits one to analyze statistically the significance of the changes in the number of each type before and after homogenizing.

In the computations percentages were used instead of numbers of bacteria because it was believed that this would give more accurate results.

The arithmetical mean of the differences in percentages of the different types of bacteria before and after homogenizing was

TABLE 12

Tabulation of the results of computation

TYPE	NUMBER OF EXPERIMENTS	AVERAGE DIFFERENCE	STANDARD DEVIATION OF DIFFERENCES	Z	ODDS
Strong acid	25	+1.93	8.68	0.2223	5:1
Weak acid	25	−0.83	20.50	0.0404	1:1
Peptonizers	25	+2.46	14.90	0.1650	2:1
Alkali and inert	25	−6.46	17.20	0.3750	1:2

determined. The standard deviation of these differences was computed and "Students" method (9) of determining the significance of the difference was used. In table 12 the results of these computations are set down.

In statistical analysis odds of less than 30:1 are not considered highly significant. In the light of this fact we can not reliably state that the percentage of any type of bacteria is changed by homogenizing. This simply indicates that even though bacteria may be added to the ice cream mix from the homogenizer that the different types are added in the same relative ratio as they already existed in the mix before homogenizing. This is not

[2] The author is indebted to Prof. L. C. Emmons for his assistance in the statistical analysis of the data.

hard to understand since the bacterial contamination in the homogenizer is due mostly to inadequate or improper cleaning of the machine after the mix has passed through.

Another point brought out by the computation is the fact that during the process of homogenizing the groups of the different types are broken up in about the same relative proportion.

GENERAL DISCUSSION

In carrying out this work an attempt was made to follow as nearly as possible factory conditions so that an actual picture would be obtained of the bacteriological changes during the process of homogenizing. In this way it was hoped to throw some light on the questions raised by the author in a previous paper and also by other workers in this field.

A close study of the data would seem to indicate that there is no definite relationship between the number of colonies on milk-powder agar and the number of bacteria found by the direct microscopic count in the ice cream mix. The type of organisms predominating in the sample might be one cause for this. If a sample had a large number of heat resistant bacteria present, a greater number would show on the plate count. A majority of the samples showed an increase in the number of colonies by the plate method after homogenizing. The same was true with the direct microscopic method. This held true both for the individuals and "groups." From a study of this data it would seem that there was some contributing cause to the increases shown.

A further study of table 2 begins to throw some light upon the cause for the increase after homogenizing. In every single case the "group" size is less. It is true that in some cases the difference is negligible. However, if one considers that the mix has been continuously agitated from forty-five minutes to an hour at a temperature of around 145°F., he will readily understand that during this agitation many of the larger clumps will have been broken up before the mix has passed through the homogenizer. That a further breaking up of clumps does occur in the homogenizer, however, is clearly indicated by the data.

Another indication that the clumps are broken up during the

process of homogenizing is indicated by the total number of groups of two or more before and after hemogenizing. In 18 or 72 per cent of the samples there is a decrease, in 5 or 20 per cent of the samples there is an increase while in 2 or 8 per cent of the samples there is no change in the total number of the groups of two or more after homogenizing as compared with the total number of groups of two or more before homogenizing. When the calculations are carried still further and the average size of the groups of two or more is calculated, we again find, as in the case of the average of "groups," that there are fewer bacteria per clump after the mix has passed through the homogenizer than before. This of course indicates that there has been a breaking up of the clumps.

When milk is passed through the homogenizer the same thing holds true. There is a gradual reduction in the size of the groups as the pressure is increased. Although the reduction is slightly irregular for the few samples run yet the sum total of the results indicates that there is a reduction in the size of the groups. The results from the few samples studied—also indicate that the homogenizer is a source of contamination as shown by the increase in the counts by both methods.

A study of the types of bacteria present in the homogenizer and present before and after homogenizing presented some interesting data. It was found that the peptonizers ran considerably higher in the water from the homogenizer than they did in the mix. This no doubt accounts for at least a part of the 5.12 per cent increase of the peptonizers found in the mix after homogenizing. The only other notable change in the percentages was in the case of the alkali and inert groups where there was a decrease after as compared to before homogenizing. The strong acid-forming and the weak acid-forming types also showed an increase after homogenizing. This may have been due partly to the breaking up of clumps and partly to the contamination from the homogenizer.

However, a statistical analysis of the significance of the slight changes in the percentages of the different types before and after homogenizing shows that they are not to be taken too seriously since the odds are not sufficient to make them highly significant.

CONCLUSIONS

1. In a majority of the cases, homogenizing increased the number of bacterial colonies in the ice cream mix as determined by the plate count using milk-powder agar.

2. In a majority of the cases, homogenizing increased the number of individuals and "groups" as determined by the direct microscopic method.

3. The average size of the "groups" and also the average size of groups of two or more in the ice cream mix are decreased. In view of the fact that all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of bacterial groups in the ice cream mix.

4. The bacterial clumps in milk are decreased in size by passing through the homogenizer. Increasing the pressure has the general tendency to decrease the average size of the groups.

5. Milk passed through the homogenizer without pressure showed an increase in colonies by the plate method and an increase in individuals and "groups" by the direct microscopic method.

6. Sterile rinse water from the homogenizer contained from 2 to 140,000 bacterial colonies per cubic centimeter.

7. The type of colonies found in the ice cream mix were strong acid-forming, weak acid-forming, peptonizers and alkali and inert.

The general conclusion that may be drawn from the data as a whole is, that, the increase in the bacteria content after homogenizing is due to two causes: First, the breaking up of bacterial clumps and, second, contamination from the homogenizer. It would therefore, seem that a part of the increase noted in the previous papers was partly apparent and partly real.

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A BIOMETRIC STUDY OF LENGTH OF METABOLISM EXPERIMENTS WITH CATTLE*

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A vital factor in all metabolism experiments is the irregularity in the voiding of excreta, and it is of obvious importance to have a definite expression of this irregularity as a measure of its influence on the accuracy of average figures. With a feed intake constant in amount there is a variation in the daily excretion of feces which is the result of all those factors contributing to the individuality of the subject.

This paper presents the results of a study of this variability, with cows and steers, as affecting the accuracy of metabolism experiments of different lengths. More specifically it is a comparison of the significance of figures representing average daily fecal outgo during periods of different numbers of days.

The weight of fresh feces excreted daily was used as a basis in this study, since a sufficient number of data on the dry basis were not available. The data for cows were taken chiefly from the original records of experiments performed at the Ohio Agricultural Experiment Station under the direction of Dr. E. B. Forbes, now director of this Institute. The results of these experiments are reported in Ohio Agricultural Experiment Station Bulletins Nos. 295, 308, 330, and 363. Through the courtesy of Director Williams of the Ohio Station we have been permitted to use the original records. Some additional data for cows were taken from the records of this Institute.

All data used were from periods in which the feed intake was maintained without variation and in which the intake had been at the same constant level for at least seven days previous to the first day of the collection period. These data comprise 1008 daily weights of feces from supermaintenance rations.

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In the study of the data a procedure similar to that of Mitchell and Grindley (1) was adopted. The weights tabulated contained five figures but it was deemed permissible and advisable to omit the last two, correcting the third for the fourth. Thus the number 27,573 was used as 276 while 26,136 was used as 261. Any error thus introduced is so slight as to be negligible.

The average figure representing the 1008 weights obtained from cow experiments was 250. Each period average was then made equal to 250 by adding to or subtracting from each weight in the period the difference between the period average and 250. As an example, in the following tabulation the number 29 is subtracted from each weight of feces in an original nineteen-day period.

ORIGINAL DATA	TRANSMUTED
279	250
261	232
276	247
294	265
249	220
280	251
284	255
272	243
269	240
297	268
276	247
294	265
289	260
296	267
283	254
288	259
264	235
280	251
275	246
Average.....279	250

It is clear that neither the individual deviations from the respective means nor consequently the standard deviation, has been changed. The object of this transmutation was to make every weight deviate from the same average, namely 250, thus

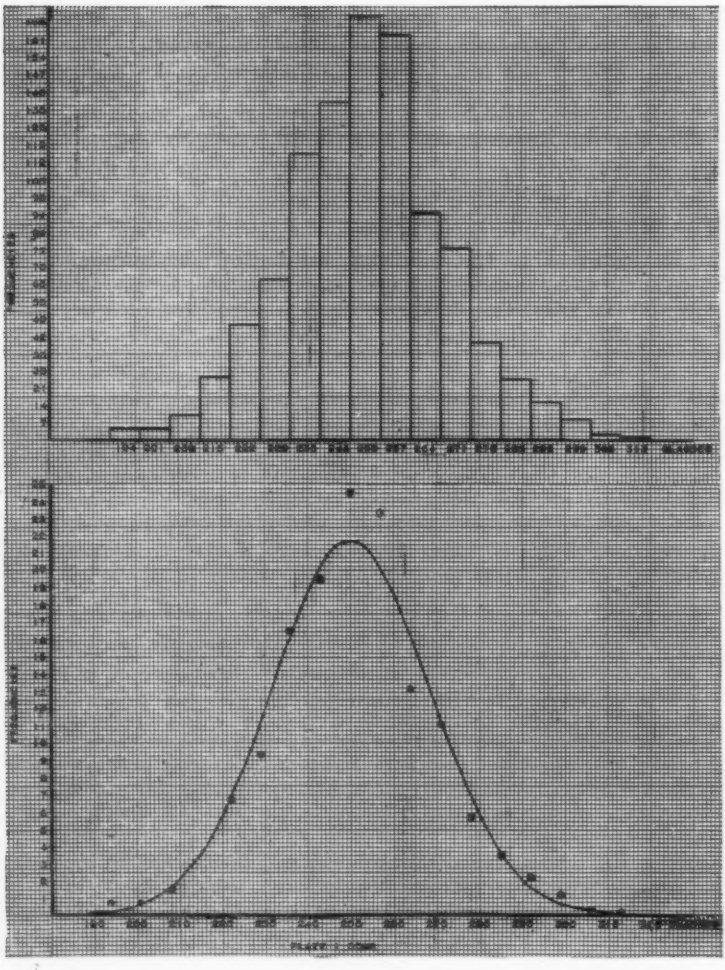

PLATE 1

eliminating the effect of the plane of feed intake on the average weight of feces for each individual period. These transmuted weights were then arranged arbitrarily, and the standard deviation from the above stated average (250) of the 1008 weights was found to be 19.1. In testing the suspicion that a few very widely divergent individual weights should be rejected, a limiting deviation was calculated according to Chauvenet's criterion (2). The weights thus rejected were respectively 176, 324, 180, 169, and 319, the limiting range being 183 to 317. This elimination changed the grand total average so slightly that it was unnecessary to determine a new one. The standard deviation of the remaining 1003 weights was 18.4.

The frequency distribution of the entire lot of weights is shown in the upper part of plate 1. This series of rectangles illustrates the actual frequency distribution of the 1003 weights. The curve drawn in the lower part of the graph is the normal frequency curve derived from the equation

$$y = \frac{n}{\sigma\sqrt{2\pi}} \times \frac{1}{e^{\frac{x^2}{2\sigma^2}}}$$

The closeness of the actual distribution to the normal curve is shown by the points enclosed by circles which are taken directly from the center points of the tops of the rectangles above. This closeness of fit is quite satisfactory.

The 1003 weights were then averaged by groups of 8, 12, 16, 20 and 24. Thus there were 125 values for eight-day period averages, 62 for sixteen-day periods and so on, there being 41 values for twenty-four-day period averages. Treating the different averages determined as variates, the standard deviation of the 125 eight-day period averages was found to be 6.42, that of the 83 twelve-day period averages 5.05, and for sixteen-, twenty-, and twenty-four-day periods the values were 3.89, 3.35, and 2.44, respectively. The average of all weights for periods of a given length was of course 250, so that the figure 6.42 represents the standard deviation of eight-day period averages around the mean 250. Collection periods of at least eight days were assumed

to be necessary for satisfactory results while twenty-four days was assumed as a practical maximum.

The following tables show the results obtained and an interpretation of them in reference to their probable errors, etc.

Taking ±4.33 as the probable error of a single eight-day period average and multiplying it by 3.2 we obtain a limiting value (right-hand column) such that the odds are 31 to 1 that any one

TABLE 1

Variability in period-average weights of cow feces

LENGTH OF PERIOD	NUMBER OF VARIATES (PERIOD AVERAGES USED)	STANDARD DEVIATION	PROBABLE ERROR OF A SINGLE VARIATE (A PERIOD AVERAGE) 0.6745 × ST. DEV. (E_s)	LIMIT OF ERROR; ODDS 31 TO 1 ($E_s \times 3.2$)
days				
8	125	6.42	±4.33	±13.86
12	83	5.05	±3.41	±10.91
16	62	3.89	±2.62	±8.38
20	50	3.35	±2.26	±7.23
24	41	2.44	±1.65	±5.28

TABLE 2

Derivation of percentage statement of error in period-average weights of cow feces

LENGTH OF PERIOD	AVERAGE	LIMIT OF ERROR; ODDS 31 TO 1	RANGE OF CERTAINTY (31 TO 1)	LIMIT OF ERROR: IN PER CENT OF MEAN (ODDS 31 TO 1)
days				
8	250	±13.86	236.14 to 263.86	5.54
12	250	±10.91	239.09 to 260.91	4.36
16	250	±8.38	241.62 to 258.38	3.35
20	250	±7.23	242.77 to 257.23	2.89
24	250	±5.28	244.72 to 255.28	2.11

of the eight-day period averages will fall within this range. This represents the manner in which the limiting values for all the periods have been obtained. In table 2 the values have been expressed in percentage.

Odds of 31 to 1 have been chosen arbitrarily as being sufficient to warrant the conclusion that when these odds exist we may be practically certain that any single variate chosen at random will

fall within these limits. This limit of error, with odds 31 to 1, stated in per cent of the mean, is computed from the limit of error in absolute terms by dividing by 250 and multiplying by 100. These percentage expressions signify the reliability of eight-, twelve-, sixteen-, twenty- and twenty-four day period averages. Expressed in words this means that the average daily fecal outgo as measured in an eight-day experiment with cows on a super-maintenance ration is not more than 5.54 per cent in error. In the case of a twenty-four-day collection period this error is not greater than 2.11 per cent.

Plate 3 illustrates graphically the different distributions of the averages of the various lengths of periods. Each curve here shown is the normal curve based on the number of variates and the standard deviation. As there were approximately three times as many eight-day periods as twenty-four-day periods the units on the ordinate were made in proportion to these lengths of periods so that each curve would enclose about the same area since it is desirable to depict the form of the curves alone. The normal curve should never quite touch the abscissa.

It was thought that there being 125 variates for eight-day period values and only 41 for twenty-four-day periods would perhaps tend toward an unfair comparison. Consequently the 125 variates were listed on pieces of cardboard, thoroughly shuffled, and 41 were drawn at random. The standard deviation of these 41 variates was found to be 5.71, with a probable error of ± 0.43. The standard deviation of the 125 variates was 6.42 ± 0.27. Since the difference between these two standard deviations is less than twice the square root of the sum of the squares of their respective probable errors, the difference is insignificant.

The data concerning steer experiments were used in a manner similar to that outlined for cows, and were taken from experiments conducted at the Pennsylvania Experiment Station and the Institute of Animal Nutrition and reported in Bulletin No. 42, United States Department of Agriculture, Bureau of Animal Industry Bulletin Nos. 51, 74, 101, and 128. In these experiments the animals were given constant amounts of feed but the plane of

intake was very close to the maintenance requirement, the variability in the elimination of feces, therefore, being characteristic of this restricted intake rather than of full feeding. The 960 weights thus obtained were treated as were the 1008 weights relating to cows, the average of the 960 being 100. These were transmuted in the manner described for the cow experiments,

TABLE 3

Variability in period-average weights of steer feces from maintenance experiments

LENGTH OF PERIOD	NUMBER OF VARIATES (PERIOD AVERAGES USED)	STANDARD DEVIATION	PROBABLE ERROR OF A SINGEE VARIATE (A PERIOD AVERAGE) 0.6745 × ST. DEV. (E_p)	LIMIT OF ERROR ODDS 31 TO 1 ($E_p \times 3.2$)
days				
8	119	3.36	±2.27	±7.26
12	79	2.81	±1.90	±6.08
16	59	2.43	±1.64	±5.25
20	47	2.12	±1.43	±4.58
24	39	1.81	±1.22	±3.90

TABLE 4

Derivation of percentage statement of limit of error in period-average weights of steer feces from maintenance experiments

LENGTH OF PERIOD	AVERAGE	LIMIT OF ERROR; ODDS 31 TO 1	RANGE OF CERTAINTY (31 TO 1)	LIMIT OF ERROR; IN PER CENT OF MEAN (ODDS 31 TO 1)
days				
8	100	±7.26	92.74 to 107.26	7.26
12	100	±6.08	93.92 to 106.08	6.08
16	100	±5.25	94.75 to 105.25	5.25
20	100	±4.58	95.42 to 104.58	4.58
24	100	±3.90	96.10 to 103.90	3.90

thus making all original period averages equal to 100. The standard deviation from the average (100) of the 960 weights was 11.75. Five weights were rejected by the method above referred to, namely, 143, 34, 153, 47, and 142, the limiting range being 59 to 141. The rejection of these weights did not significantly change the average of the remaining 955. The standard deviation of these 955 weights was 11.17. The actual frequency

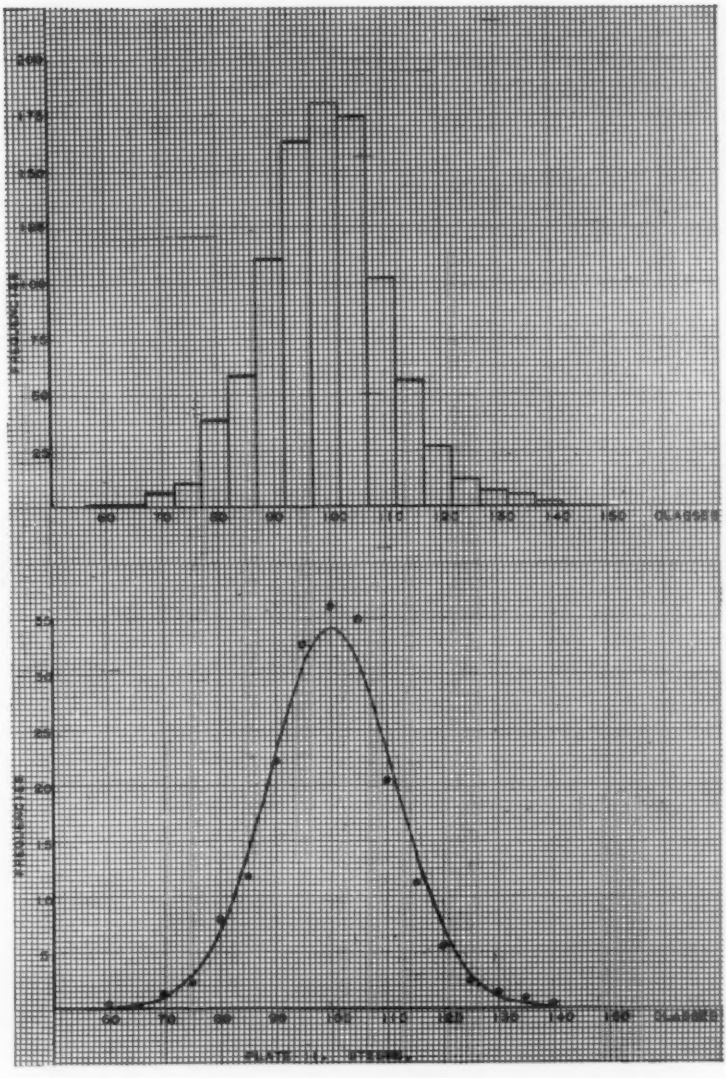

PLATE 2

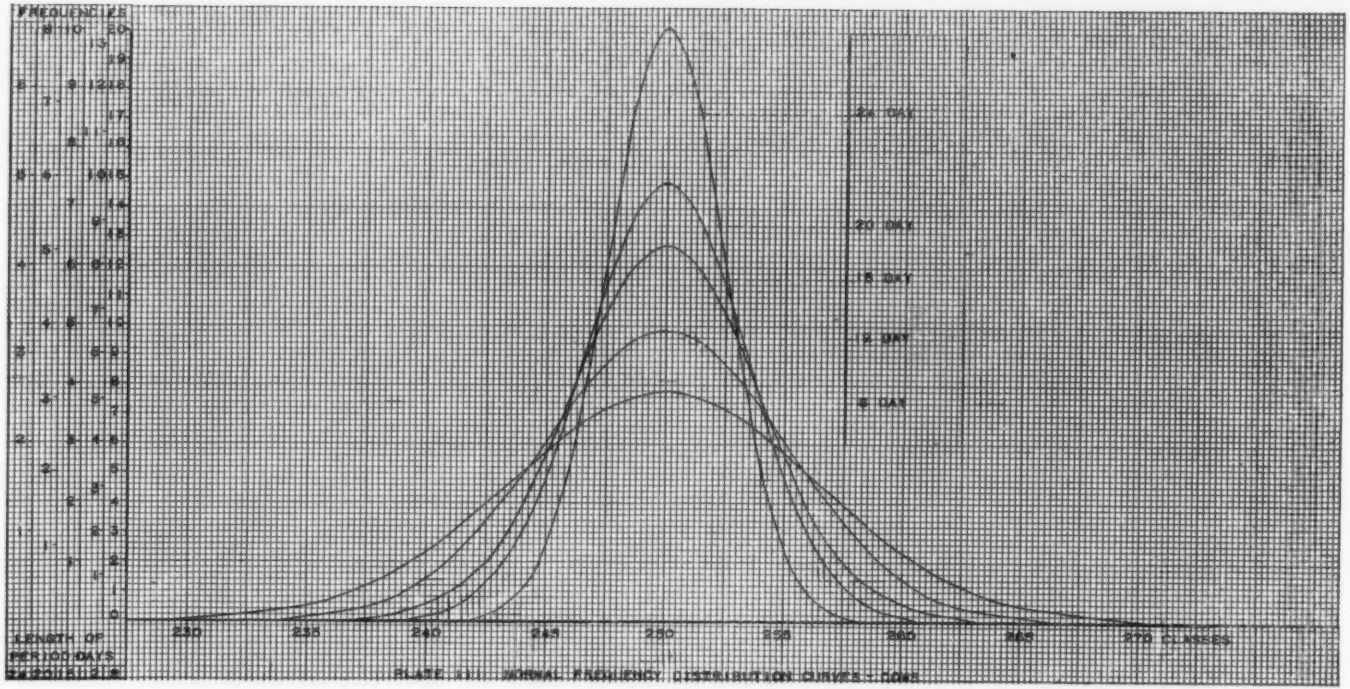

PLATE 3: NORMAL FREQUENCY DISTRIBUTION CURVES - DOSE

PLATE 3

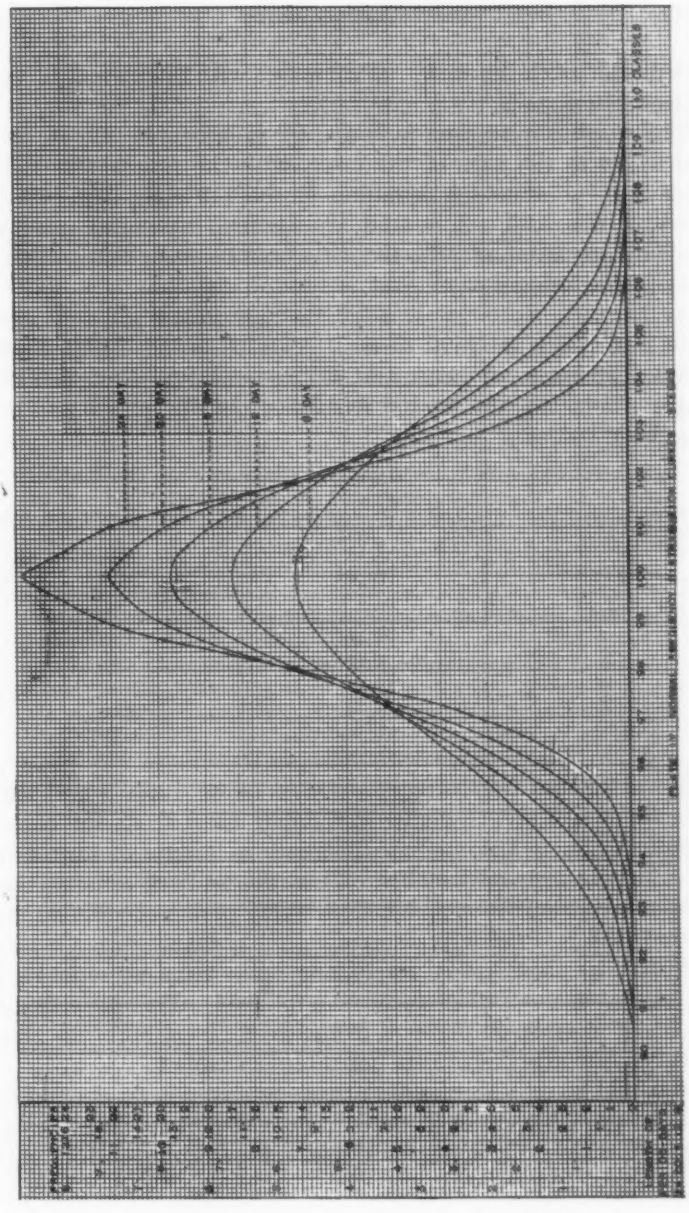

PLATE 4

distribution of the entire lot, as compared with the normal curve, is shown in plate 2.

The weights were then averaged by groups of 8, 12, 16, 20, and 24, as in the case of the cows, with the results shown in table 3. The standard deviation of the 119 eight-day period averages was 3.36. A second arrangement by eights gave a standard deviation of 3.59, the difference between this and the above value being insignificant.

As the mean in this case was 100, the same values will appear in table 4 in which the limit of error is stated in per cent. Thus 100 ± 7.26 means of course 100 ± 7.26 per cent. Plate 4 illustrates graphically the normal frequency distributions based on the above values.

TABLE 5

Percentage statement of limits of error in period-average weights of cow and steer feces

LENGTH OF PERIOD	STEERS	COWS
days		
8	7.26	5.54
12	6.08	4.36
16	5.25	3.35
20	4.58	2.89
24	3.90	2.11

It will be seen from a comparison of tables 2 and 4 that the data for steers give a higher variability than those for cows, doubtless due to the marked difference in planes of nutrition prevailing in the experiments. Thus the limit of error of the average weight of feces from a sixteen-day period experiment on steers which received approximately a maintenance ration is but little smaller than the corresponding value from an eight-day experiment on cows which received a full feed. For convenience the limits of error, in per cent of the mean, from both the cow and the steer experiments are assembled in table 5.

It should be remembered that these percentages do not represent the probable errors of these lengths of periods, but signify rather that the odds are 31 to 1 that the percentage errors will

not be in excess of these values. They express the degree of reliability of the period-average feces weights from experiments with cows on full feed and steers on an approximate maintenance basis.

SUMMARY

From a statistical study of 1008 weights of cow feces, and 960 weights of steer feces, resulting from metabolism experiments of varying length but constant feed intake, it is shown that with cows the chances are 31 to 1 that with collection periods varying in length from eight days to twenty-four days the errors of the averages will not exceed 5.54 to 2.11 per cent; and that with steers on restricted feed intake, the chances are, similarly, that with collection periods of eight to twenty-four days the errors of the averages will not exceed 7.26 to 3.90 per cent.

The experimenter will be his own judge as to the degree of variability permissible in a particular piece of research.

ACKNOWLEDGMENTS

I wish to express my thanks to Director E. B. Forbes of this Institute for suggesting this problem to me, to Director C. G. Williams, of the Ohio Agricultural Experiment Station for the courtesy of the loan of original records, and to W. W. Braman for helpful suggestions throughout this study.

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A COMPARISON OF THE METHYLENE BLUE REDUCTASE TEST AND THE DIRECT MICROSCOPIC COUNT IN GRADING MILK AT MILK PLANTS*

H. C. TROY

Department of Dairy Industry, Cornell University, Ithaca, New York

During recent years the methylene blue reductase test has been advocated as a practical method to use for determining the approximate number of bacteria in milk. The test has been used quite extensively in some European countries and recently to some extent in this country at dairy manufacturing plants and milk receiving stations.

The well established plate count method and the more recent direct microscopic count method have been adopted as official by the American Public Health Association. These tests are more expensive and complicated and demand more care and skill than is required in operating the reductase test. But the latter test has not been used in this country to such an extent that its place in the dairy industry has been definitely established.

During the months of July, August, and September, 1924, a number of dairy manufacturing plants and milk receiving stations were visited by the writer and a sample of the milk delivered by each producer was tested for bacteria by both the methylene blue reductase test and the direct microscopic method. The object of the work was first, to determine the grade (A, B or C) of each producer's milk on the day it was tested and, second, to learn how the results obtained by the reductase test checked with those obtained by the direct microscopic count method.

METHOD OF PROCEDURE

The operator of the tests visited each plant once and remained there from two days to one week, the time varying according to the number of samples tested. The apparatus used in making

*Received for publication January 24, 1925.

the reductase test was an outfit obtained from a dairy supply concern. In some instances samples of the night's milk only were tested. In others, samples of both the night's and morning's milk were taken and tested separately; but the usual practice was to take a small amount of the milk from each can of the night's

TABLE 1

Summary of data in the comparison of the methylene blue reductase test and the direct microscopic count in grading milk at milk plants

PLANT NUMBER	NUMBER OF SAMPLES	REDUCTASE TEST			MICROSCOPIC COUNT			NUMBER IN SAME GRADE BY BOTH METHODS	NUMBER PLACED IN DIFFERENT GRADES	NUMBER IN A LOWER GRADE BY REDUCTASE TEST	NUMBER IN A LOWER GRADE BY MICROSCOPIC COUNT	PERCENTAGE AGREEMENT
		Grade A	Grade B	Grade C	Grade A	Grade B	Grade C					
1	94	71	9	14	60	18	16	68	26	10	16	72.2
2	87	67	10	10	59	15	13	68	19	4	15	78.1
3	68	44	4	20	36	8	24	58	10	0	10	85.3
4	95	44	15	36	44	19	32	83	12	4	8	87.3
5	200	135	24	41	136	23	41	170	30	12	18	85.0
6	100	72	13	15	72	12	16	82	18	8	10	82.0
7	225	122	20	83	125	35	65	182	43	24	19	80.8
8	100	72	11	17	72	10	18	93	7	3	4	93.0
9	100	80	6	14	78	6	16	86	14	5	9	86.0
10	100	86	7	7	83	12	5	87	13	1	12	87.0
11	100	72	8	20	65	14	21	85	15	3	12	85.0
12	100	83	6	11	74	14	12	89	11	1	10	89.0
13	98	84	4	10	84	6	8	94	4	3	1	96.0
14	96	80	5	11	72	11	13	86	10	1	9	89.5
15	50	47	1	2	42	6	2	46	4	0	4	92.0
16	84	80	4	0	77	5	2	79	5	0	5	94.0
17	100	80	14	6	73	18	9	88	12	1	11	88.0
18	150	125	6	19	118	13	19	137	13	4	9	91.3
Totals....	1,947	1,444	167	336	1,370	245	332	1,681	266	84	182	86.3

and morning's milk that a producer delivered in the morning and place these different portions together in a sterile bottle to serve as a sample of his milk. The samples, usually, were iced in a tray when taken. When the desired number were obtained the tray of samples was taken to the laboratory in the plant.

The reductase test and the direct microscopic count were made as soon after the samples were obtained and as rapidly as the work could be done conveniently.

In the reductase test the tubes in the incubator were held in a water bath at 100° to 105°F., usually 103°F. and were examined at the end of the first hour and at half-hour intervals thereafter up to five hours. When the time required to reduce the methylene blue color was more than three hours, the milk was classed as Grade A. When the time was three hours or more than two hours, it was classed as Grade B. When the time was two hours or less, it was classed as Grade C. This basis of grading was advised by the firm that placed on the market the apparatus for making the test. The concentration of methylene blue solution supplied with the apparatus was such that it gave 1 part of the dry crystalline dye to 200,000 parts of milk in the test.

In making the direct microscopic count single bacteria and clusters were each counted as one source. But when a smear showed few single bacteria or pairs and relatively large numbers of clusters some allowance was made for the larger number of bacteria in the clusters.

All samples of milk containing less than 300,000 sources per cubic centimeter by the direct microscopic count were classed as Grade A. Samples containing 300,000 sources and upwards to 1,500,000 were classed as Grade B. All samples that contained 1,500,000 bacteria per cubic centimeter or over that number were classed as Grade C.

The correlation between the two methods was 86.2 per cent. It was highest at the plants that received the largest proportions of milk of Grade A quality.

The microscopic count placed more samples in lower grades than were so placed by the reductase test. There were 266 samples, out of 1947 samples tested, for which the methods did not agree as to the Grade in which the milk should be placed. Of these 266 samples, 182 were placed in a lower grade by the direct microscopic count method, whereas, only 84 samples were placed in a lower grade by the reductase test.

In those instances where the two tests did not agree, the milk, usually, was placed in adjoining grades, but there were a few instances where milk was placed in the highest grade by one method and in the lowest grade by the other. Some of these instances may have been caused by errors in technique but probably not all of them.

DAIRY NOTES

MORE HIGH GRADE ARTICLES NEEDED

Nearly all manuscripts approved by the editorial board have now been published in the JOURNAL OF DAIRY SCIENCE. From this time on we hope to be able to publish articles shortly after they have gone through the regular channels.

The editorial board is very anxious to give space only to high grade articles and to preserve a happy balance of material of interest to the production side as well as those interested largely in the manufacturing divisions of the dairy industry.

May I not urge that all persons having available manuscripts forward them to the editor at once. Help us to improve the scope and quality of the JOURNAL by sending us your *best* papers *now* while the material is up to date and timely.

J. H. FRANDSEN,
Editor.

DAIRY SCIENCE NOTES

The headquarters for the Dairy Science Association during the National Dairy Exposition at Indianapolis will be the Hotel Severin. This hotel is reserving a block of rooms for the members of the association and those of you interested in obtaining a reservation should do so at once. These rooms will be held for us a short time after which those not filled will be cancelled. The best rates that could be obtained at this or any other hotel was from $5 to $7 per double room, all rooms with bath. In writing to this hotel mention the fact that you are a member of the American Dairy Science Association.

The following men have been designated as a committee in the Intercollegiate Dairy Cattle Judging Contest:

W. W. Swett, *Chairman*
H. H. Kildee
A. A. Borland
L. H. Fairchild
L. W. Ingham
J. W. Fuller
William Regan
J. B. Fitch

The members of committees of Section III, the Extension section, are as follows:

Dairy Manufacturing
J. D. Brew, Ithaca, N. Y.
A. W. Rudnick, Ames, Iowa
G. N. Tobey, Knoxville, Tenn.

Feeding
E. B. Fitts, State College, Pa.
C. B. Finley, Ames, Iowa
W. J. Keegan, Clemson College, S. C.
A. R. Morrill, Storrs, Conn.
H. A. Hopper, Ithaca, N. Y.

Milk Campaigns
Jessie M. Hoover, U. S. Bureau of Dairying, Washington, D. C.
W. P. B. Lockwood, Amherst, Mass.
Dorothy S. Bulkley, Storrs, Conn.
Mary E. Thomas, Baton Rouge, La.

Bull Associations
L. A. Higgins, Starkville, Miss.
G. G. Cushman, Clemson College, S. C.
M. J. Regan, Columbia, Mo.
A. R. Merill, Storrs, Conn.

Calf Clubs
E. J. Perry, New Brunswick, N. J.
E. M. Harmon, Columbia, Mo.
C. A. Hutton, Knoxville, Tenn.
R. H. Olmstead, State College, Pa.

Cow Testing Associations
A. C. Baltzer, East Lansing, Mich.
A. J. Cramer, Madison, Wis.
E. A. Hanson, St. Paul, Minn.
C. R. Gearhart, State College, Pa.
L. P. Emmerick, Blacksburg, Va.

J. B. FITCH,
Secretary.

HYDROCYANIC ACID RETAINED BY FUMIGATED CHEESE

Messrs. E. R. de Ong and C. L. Roadhouse have recently published the recommendation that cheese may be fumigated with hydrocyanic acid gas for the destruction of certain cheese pests ("Cheese Pests and Their Control," Calif. Exp. Sta. Bul. 343, May, 1922). The ability to use this gas with safety in cheese rooms without removing the cheese is much to be desired and in connection with his work with the cheese

skipper (Piophila casei L.) the writer made several preliminary tests of the advisability of using this fumigant. He is indebted to Dr. R. E. Holm, division of Virus-Serum Control, Bureau of Animal Husbandry, U. S. D. A., for the analyses of the fumigated cheese. A review of the experiments and results follows.

October 10–11, 1921. Fumigating box, 100 cubic feet. Charge 4 ounces NaCN, 6 ounces H_2SO_4, 8 ounces water. Temperature at start about 55°F.; relative humidity about 80 per cent. Exposure of cheese 10:30 a.m. to 8:45 a.m. Cheese tested: Camembert, Cheddar and Swiss, the pieces being roughly 3 by 3 by 3 inches. One side of each piece freshly cut and marked. Gas not strong when box opened. Analysis showed considerable HCN in the cheese, especially in the Camembert; least in the Cheddar. The gas in the cheese much reduced after airing about one day.

October 17–18, 1921. Fumigating box the same, but repaired to reduce leakage. Charge 2 ounces NaCN, 3 ounces H_2SO_4, 4 ounces water. Temperature about 63°F.; relative humidity 89 per cent. Exposure of cheese 3:00 p.m. to 9:30 a.m. Gas strong when box opened. Cheese tested, same as in first trial. Analyses showed that a large amount of acid was retained, even after airing for about one day. After this airing, more acid was present in the center of the cheese near the surface, due probably, to slower dissipation from within.

October 18–19, 1921. Fumigating box the same. Charge 1 ounce NaCN; 1½ ounces H_2SO_4, 2 ounces water. Temperature about 65°F.; relative humidity 80 per cent. Exposure of cheese 11:30 a.m. to 10:00 a.m. Temperature steadily declined to 55°F. at end of trial. Migrant skipper larvae used as indicators of efficiency of the fumigation were killed; these were exposed in Petri dishes. Cheese aired for 24 hours before being sent to Dr. Holm's laboratory. Analyses showed that HCN was found in all the samples, but the concentration in the Cheddar amounted to a mere trace.

On account of the tenacity with which skipper larvae cling to life in the presence of hydrocyanic acid gas, especially when embedded in their food materials, the writer feels that dosages up to and including one ounce per 100 cubic feet would be largely wasted. Cheese mites also are reported to be very resistant to this gas. It would not be safe to assume that all cheeses in a given room were protected by an unbroken coating of paraffin, and in view of the very evident tendency of cheese to retain this poisonous acid and the fact that cheese is not usually cooked before being eaten (a process which would be expected to aid the

volatilization of the retained acid), the writer feels that until more extensive tests are made to prove otherwise, in retard to each variety of cheese, cyanide fumigation of cheese should not be recommended.

PEREZ SIMMONS,
Entomologist, Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture.

FOURTH ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Reported by J. A. Gamble

The meeting was called to order by the Chairman, J. P. LaMaster, at Atlanta, Ga., February, 1925, with 21 Southern Dairy workers present.

Professor LaMaster, in his presidential address, covered the developments in dairy husbandry in the Southern States during the past four years and made suggestions for the guidance of the meeting.

The first speaker was J. H. McClain of the United States Dairy Bureau who outlined the Projects in Dairy Husbandry which should have the attention of the dairy workers in the different parts of the South and made some suggestions for inaugurating and carrying forward this work. These suggestions were discussed by Mr. Oliver of the American Jersey Cattle Club, Mr. Humphries of Georgia, J. S. Moore of Mississippi, and V. M. Williams of North Carolina.

Professor Wylie delivered an outstanding address supported by charts on "The Age, Season and Lactation Factors in Milk Production." This was one of the outstanding contributions to the meeting.

Mr. Oliver of the American Jersey Cattle Club addressed the convention advocating the formation of more Boy's Calf Clubs and the necessity of starting new breeders right.

Mr. Stanley Combs of the Holstein Friesian Association gave an inspiring talk on the "Benefits resulting from the Introduction of Pure Bred Cattle into the South" and made a special plea for the formation of Cow Testing Associations, Bull Associations and Calf Clubs. He made the point that in bringing better dairying into the South, we must reach the man with a few cows. He stated that he was working for the advancement of dairying in the South first, knowing that as a result of such work the Holstein Friesian would come into her proper place.

Mr. Humphries of the Southern Railroad was called on and gave a

splendid talk relating to the Need for Increasing the Production of Cows and Increasing the Care in Maintaining Quality, suggesting Cow Testing Associations and Bull Associations as a means for this end. He further suggested that the dairy workers in each of the Southern States come together at least once a year and discuss their State problems.

Mr. McWorter of the Georgia Railroad discussed the work his road was doing in bringing about improved pastures in the State of Georgia and the allied work which has been carried forward continuously by his Company for the past several years.

L. C. Wells of the Experiment Station, Alabama, in discussing his dairy problems said that their effort was to get a few dairy farms on a profitable basis in Alabama where better feeding and better breeding could be pointed to as an example for others to follow. He was of the opinion that such men would do more to place dairying on a foundation from which it can grow prominently then efforts less enduring.

Mr. McCracken of the L & N Railroad discussed the interest of his company in Calf Club Work and suggested that they might be induced to support the annual Dairy Cattle Judging Contest held at the Tri-state Fair, Memphis.

The report of the Dairy Cattle Judging Contest, submitted by Chairman J. J. Hooper was read by the Secretary and discussed by several members of the committee. It was the concensus of opinion that the Tri-state Judging Contest should be supported by all members of the Southern Division of the American Dairy Science Association.

Mr. Wylie suggested that the Chairman, J. J. Hooper, get in touch with the teachers in Dairy Cattle Judging early in the spring and continue to push the interest in the affair urging also that Kentucky be represented. The report of Chairman Hooper was accepted with thanks and the committee continued.

The report of the Resolution Committee was read and adopted.

Resolution of the Dairy Husbandry Section

The Committee on resolutions for the Southern Division of the American Dairy Science Association recommend the following:

I. That we urge the active efforts of all agencies interested in Southern Agriculture toward the furtherance of the eradication and control of diseases detrimental to the progress of the dairy industry, some of the more important being tuberculosis and contagious abortion. Especial effort is urged toward the task of completing the eradication of the cattle tick.

II. That we strongly urge the greater consumption of dairy products from the standpoint of growth and development of the young and proper nourishment of all, the fostering of efforts now being put forth toward this end is solicited.

III. In view of the progress dairy farming has made during the past few years of stress in the cotton belt and in view of the further fact that there are great possibilities for development in this industry in the South, we ask a careful study of the relation that the dairy industry should bear to a well rounded system of agriculture to the end that all interested in Southern Agricultural development may lend a hand in a unified way in the furtherance of such a system.

IV. That the active and closest coöperation of the agronomy workers of the South is needed and earnestly solicited in behalf of crops and pastures best suited for producing feed for dairy cattle. Without the basic principle of cheap home grown feed dairying cannot reach its most profitable development and help is needed to establish the proper cropping systems for various soil types in the South.

V. That the Southern Division of the American Dairy Science Association wishes to express its appreciation and thanks for the aid of public spirited organizations in the support of constructive projects for the development of dairying in the South. We urge unified effort in the formation and execution of policies for further work in behalf of the industry.

Second session

The Extension Committee report was presented by C. A. Hutton, was discussed at length and adopted.

L. S. Edwards of Tennessee delivered the report of the Dairy Manufacturing and Marketing Committee. This showed splendid work and was much appreciated by those present. The report was adopted.

Report on Official Testing, by J. S. Moore of Mississippi was listened to with a great deal of interest, discussed fully and adopted.

The report of the Nominating Committee was next received and adopted as follows:

Chairman: C. A. Hutton, Tennessee; J. S. Moore, Mississippi.

Vice-Chairman: J. A. Gamble, Maryland; F. W. Fitch, Georgia.

Secretary-Treasurer: J. P. LaMaster, South Carolina; J. S. Arey, North Carolina.

In the absence of A. C. Baer, the Secretary read his paper on "Southern Dairy Manufacturing."

The report of progress dealing with the work of the Southern Livestock Research Board was read by V. M. Williams of North Carolina.

J. A. Gamble discussed the work of the College Feed Conference Board and called attention to the fact that the next meeting was to be held in New York on February 27th and 28th suggesting that it might be of interest to several present.

The report on Animal Breeding by Dr. Lush was read by the Secretary.

Professor Barre, Secretary of the Association of Agricultural Workers being present was introduced and after complimentary remarks asked that all of the papers delivered before the Convention be abstracted so that they might be printed among other section material in the annual report of the Association of Southern Agricultural Workers.

J. A. Gamble reported the latest developments relative to changes in the constitution of the American Dairy Science Association which would permit the formation and affiliation of Junior Dairy Clubs.

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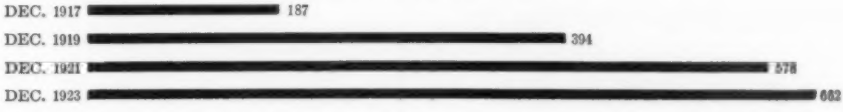

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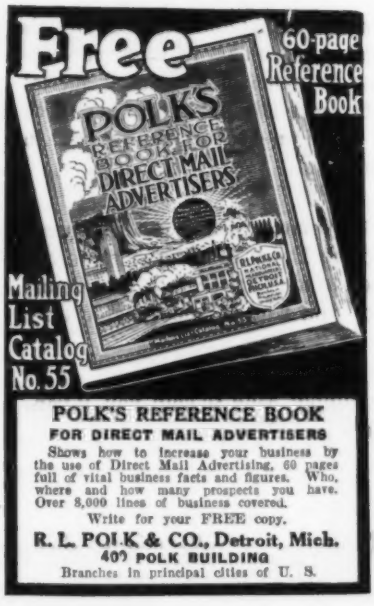

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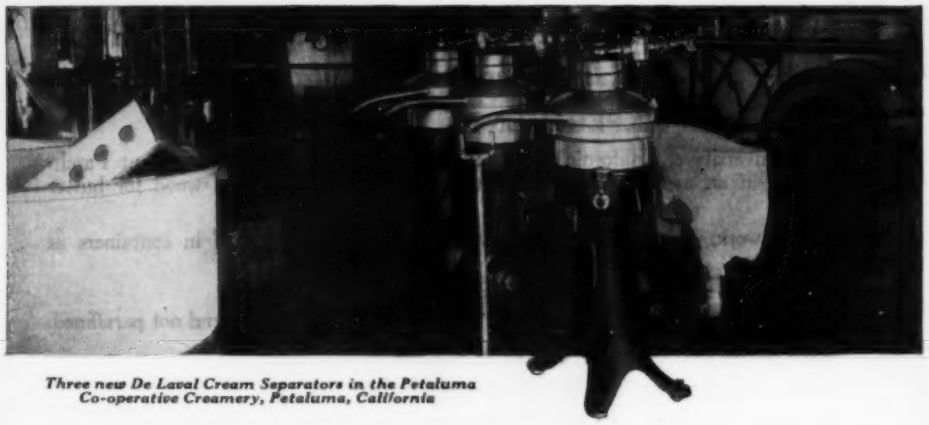

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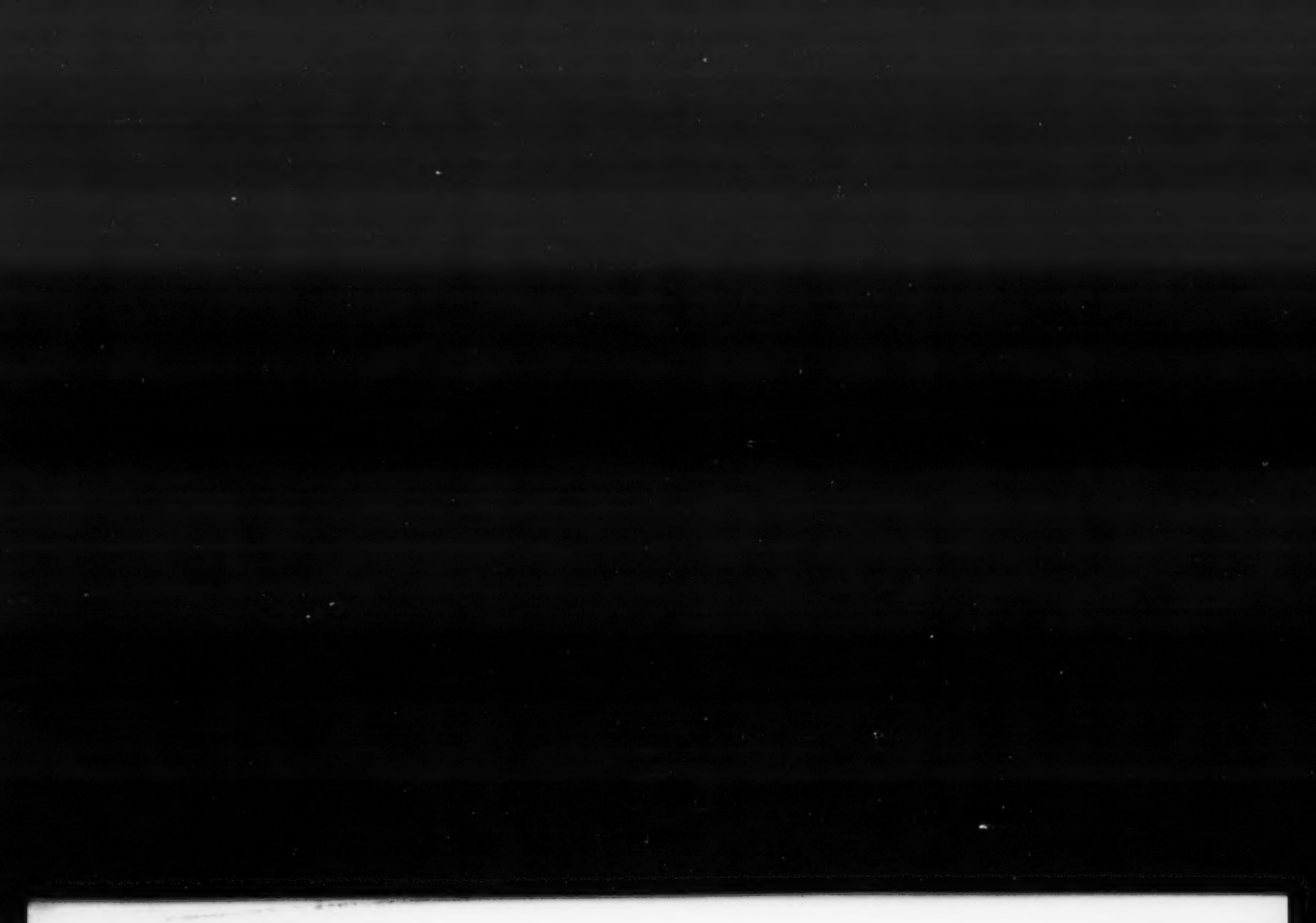

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